

Discrepancy between Measured Serum Total Carbon Dioxide Content and Bicarbonate Concentration Calculated from Arterial Blood Gases

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Disclosures can be found in Additional Information at the end of the article

Abstract

Large differences between the concentrations of serum total carbon dioxide (TCO₂) and blood gas bicarbonate (HCO₃⁻) were observed in two consecutive simultaneously drawn sets of samples of serum and arterial blood gases in a patient who presented with severe carbon dioxide retention and profound acidemia. These differences could not be explained by the effect of the high partial pressure of carbon dioxide on TCO₂, by variations in the dissociation constant of the carbonic acid/bicarbonate system or by faults caused by the algorithms of the blood gas apparatus that calculate HCO₃⁻. A recalculation using the Henderson-Hasselbach equation revealed arterial blood gas HCO₃⁻ values close to the corresponding serum TCO₂ values and clarified the diagnosis of the acid-base disorder, which had been placed in doubt by the large differences between the reported TCO₂ and HCO₃⁻ values. Human error in the calculation of HCO₃⁻ was identified as the source of these differences. Recalculation of blood gas HCO₃⁻ should be the first step in identifying the source of large differences between serum TCO₂ and blood gas HCO₃⁻.

Categories: Emergency Medicine, Internal Medicine, Nephrology

Keywords: total co2 concentration, bicarbonate concentration, carbonic acid/bicarbonate pk', acid-base status, acidemia, hypercapnia, respiratory acidosis, metabolic acidosis

Introduction

The measured total carbon dioxide (TCO₂) in serum has three main components, bicarbonate anion (HCO₃⁻), dissolved carbon dioxide (dCO₂), and carbonic acid (H₂CO₃), which is the hydrated form of carbon dioxide. The concentration of dCO₂ is calculated from the partial pressure of CO₂ (pCO₂). Not taking into account the concentration of H₂CO₃ in this calculation is the source of a negligible error. In body fluids, one H₂CO₃ molecule is at equilibrium with 340 molecules of dCO₂ under normal temperature and physiologic conditions [1]. At a pH of 7.40, the concentration of HCO₃⁻ is 20-fold higher than the concentration of dCO₂ and the

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concentration of H_2CO_3 is 1.2/340 or 0.004 mmol/L when the TCO_2 concentration is 25.2 mmol/L and the HCO_3^- level is 24 mmol/L. The ratio $\text{HCO}_3^-/\text{dCO}_2$ decreases progressively as the pH decreases but remains routinely high. Consequently, the concentrations of TCO_2 in a serum sample and of HCO_3^- in a simultaneously drawn blood gas sample should differ only slightly, with TCO_2 typically exceeding HCO_3^- by less than 3 mmol/L.

In a sample of blood gases, the concentration of dCO_2 is expressed by multiplying the pCO_2 by a proportionality coefficient S converting units of partial pressure to units of molar concentration. The value of HCO_3^- in blood gases is routinely obtained by entering the measured values of pH and pCO_2 in an algorithm representing the Henderson-Hasselbach equation, the general expression of which is as follows:

$$\text{pH} = \text{pK}' + \log(\text{HCO}_3^-/[\text{SxpCO}_2]) \quad \{1\}$$

where pK' is the negative logarithm of the first dissociation constant of H_2CO_3 . The general expression of HCO_3^- concentration is obtained by rearranging equation 1 as follows:

$$\text{HCO}_3^- = \text{SxpCO}_2 \times 10^{(\text{pH} - \text{pK}')} \quad \{2\}$$

The coefficient S is not constant. The temperature and composition of the solution tested are factors affecting this coefficient. At body temperature, the coefficient S obtains the value 0.0301 mmol/L per mm Hg in blood [1]. The pK' of H_2CO_3 in aqueous solutions is around 3.5 [2]. The calculation of this pK' in biological fluids assumes that H_2CO_3 consists of the whole dCO_2 . Therefore, the apparent value of pK' is at or very close to 6.1 under normal conditions [1].

Large differences between simultaneously obtained concentrations of serum TCO_2 and arterial blood HCO_3^- are encountered in certain instances. These differences complicate the evaluation of the acid-base status of patients and may lead to diagnostic and therapeutic errors. One potential source of the discrepancy is rooted in the erroneous assumption that the pK' of H_2CO_3 is constant at 6.1. It was suggested that this pK' is the cause of erroneous calculation of HCO_3^- in blood gases [3].

Differences in the concentrations of closely obtained TCO_2 and HCO_3^- may have other origins in addition to a wrong value of pK' applied in the calculation of HCO_3^- . A systematic search for the cause of these differences is merited. We report a patient presenting with a large difference between serum TCO_2 and arterial blood gas HCO_3^- in two consecutive sets of serum and blood gas samples. By our calculations, the pK' was not the cause of this difference. A different reason for the difference was detected and led to the correct diagnosis of the acid-base disturbance, which was in doubt because of the $\text{TCO}_2/\text{HCO}_3^-$ discrepancy.

Case Presentation

Patient

Permission to report this case was obtained from the Raymond G Murphy VA Medical

Center Institutional Human Research Committee. This paper was approved as a case report with waiving of the informed consent with the proviso that all identifying information was removed from the text.

A 61-year-old man with acute confusion and shallow and infrequent respirations was transferred to this hospital from a nursing home. He carried the diagnosis of alcoholic cirrhosis with ascites. One year prior to this admission, he had a small bowel resection with ileostomy. Six months later, he had surgical repair of an incarcerated inguinal hernia.

In the days prior to admission, he had consumed an unknown number of oxycodone tablets. On admission, his temperature was 36.9° C, blood pressure - 132/68 mm Hg, and pulse rate - 89 per minute. Urine toxicology revealed large concentrations of opioids. Acute respiratory failure was diagnosed. He underwent tracheal intubation and was ventilated. Table 1 shows arterial blood acid-base parameters and serum TCO₂ in the first two sets of simultaneously drawn samples of arterial blood gases and serum. Arterial blood gas values were determined in a satellite “point-of-care” instrument almost immediately after collection of the samples. The concentration of serum TCO₂ exceeded that of arterial blood gas HCO₃⁻ by 10.7 mmol/L in the first set of blood samples and by 7.6 mmol/L in the second set. In the first and second serum samples, respectively, sodium concentrations were 139 and 138 mmol/L, chloride concentrations were 102 and 103 mmol/L, and anion gaps were 8 and 9 mEq/L. Lactate level was 1.1 mmol/L in the first blood sample.

Study	Arterial pH	Arterial pCO ₂ mm Hg	Arterial HCO ₃ ⁻ mmol/	Serum TCO ₂ mmol/L
1st	6.91	149	18.3	29.0
2nd	7.11	74	18.4	26.0

TABLE 1: Reported simultaneously obtained serum and arterial blood gas acid-base parameters

The patient’s respiratory acidosis improved rapidly with ventilation. He was extubated the next day. His mental status improved slowly and returned to baseline in four days. The concentration of serum TCO₂ was between 24 and 27 mmol/L in samples obtained after the first two samples during this hospitalization.

Investigations of the source of the discrepancy between TCO₂ and HCO₃⁻

Identification of the source of the difference between serum TCO₂ and arterial blood HCO₃⁻ was attempted in three successive steps:

The Potential Effect of Elevated pCO₂

The concentration of TCO₂ is considered at first approximation to be equal to the sum of the

concentrations of $d\text{CO}_2$ and HCO_3^- . Because it is a linear function of $p\text{CO}_2$, $d\text{CO}_2$ accounts for progressively larger differences between serum TCO_2 and HCO_3^- at progressive levels of hypercapnia. We tested whether $d\text{CO}_2$, calculated as $0.0301 \times p\text{CO}_2$, accounted for the differences between serum TCO_2 and blood gas HCO_3^- in our patient. Figure 1 shows this comparison for the first set of measurements. The figure shows the sum of blood gas HCO_3^- , $d\text{CO}_2$, and H_2CO_3 in an idealized normal subject with an HCO_3^- of 24 mmol/L and a $d\text{CO}_2$ of 1.2 mmol/L and in the first set of arterial blood gases in the patient of this report, plus the measured TCO_2 in the first blood sample of this patient.

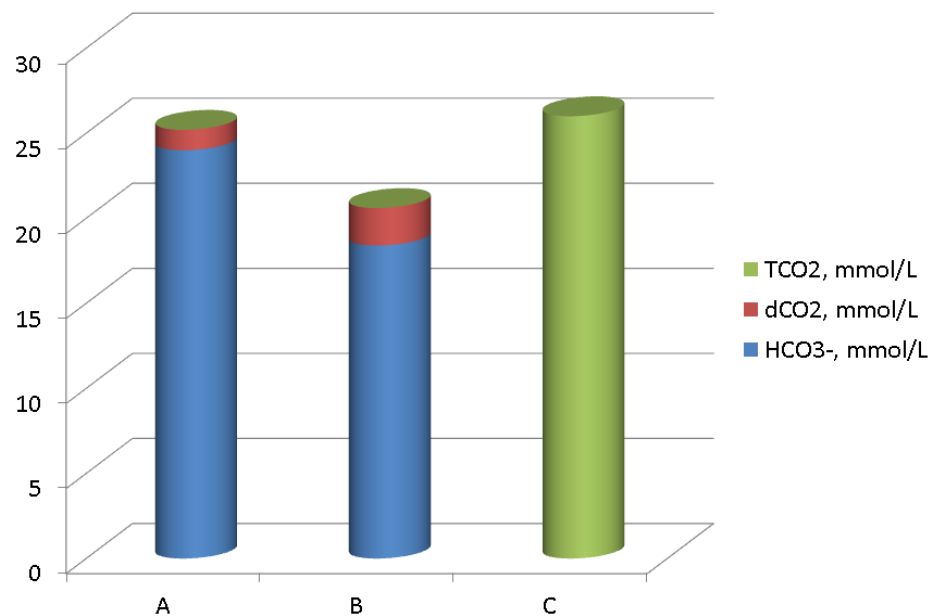


FIGURE 1: Components of total carbon dioxide content

A: Idealized normal subject. B: Arterial blood gas $d\text{CO}_2$, plus H_2CO_3 , plus HCO_3^- in the first set of blood tests in the patient presented in this report. C: Serum TCO_2 in the first set of blood tests in the patient of this report. The concentration of arterial $d\text{CO}_2$ was substantially higher in B than in A. Despite adjustment for the high $d\text{CO}_2$, the measured concentration of TCO_2 in serum (C) remained substantially higher than its computed concentration in the blood gasses (B).

Figure 2 shows the comparison between the sum of blood gas HCO_3^- , $d\text{CO}_2$, and H_2CO_3 , plus the measured serum TCO_2 in the second set of measurements and in the idealized normal subject.

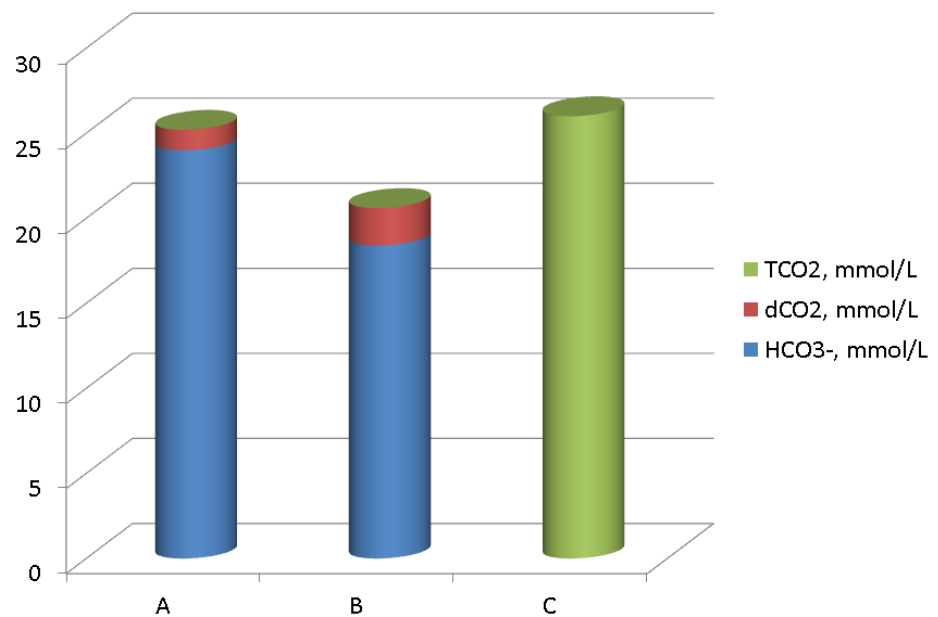


FIGURE 2: Components of total Carbon dioxide content

A. Idealized normal subjects. B. Arterial blood gas HCO₃⁻, plus dCO₂, plus H₂CO₃ in the second set of blood tests in the patient of this report. C. Measured serum TCO₂ in the second set of blood tests in the patient of this report. The measured serum TCO₂ (C) remained substantially higher than the calculated blood gas TCO₂ (B).

Figures 1 and 2 have two noticeable findings. The first finding is that the concentration of H₂CO₃ was truly negligible. The concentration of H₂CO₃ should be shown in red. Red colour columns did not appear in the figures even at a pCO₂ of 149 mm Hg. The main finding of Figures 1 and 2 is that accounting for dCO₂ reduced the difference of the concentrations between serum TCO₂ and arterial blood HCO₃⁻ but did not eliminate it. Substantial parts of this difference remained unaccounted for.

Potential Effect of Changing pK'

The pK' of H₂CO₃ in various solutions is not a fixed value. Variables affecting this pK' include the temperature, acidity, and ionic strength of the solution tested [3-7]. The first two variables have been studied extensively [3-6]. The pK' value increases with increasing acidity and decreasing temperature. Increasing the pK' value in equation 2 lowers the value of HCO₃⁻ calculated from it for the same value of pCO₂. In the chapter by Madias and Cohen [1], Table 1-3 shows apparent pK' values in human plasma at temperatures between 10 and 40° C and pH values between 7.0 and 7.6. In this table, the lowest plasma pK', at a pH of 7.60 and a temperature of 40° C, was 6.077 while the highest pK', at a pH of 7.00 and a temperature of 10° C, was 6.246. Figure 3 shows the values of HCO₃⁻ calculated from equation 2 for a pCO₂ of 149

mm Hg and pK' values of 6.1, 6.077, and 6.246. The figure shows substantial differences between these values. The lowest value of HCO_3^- was calculated for a pK' of 6.246 and the highest value for a pK' of 6.077.

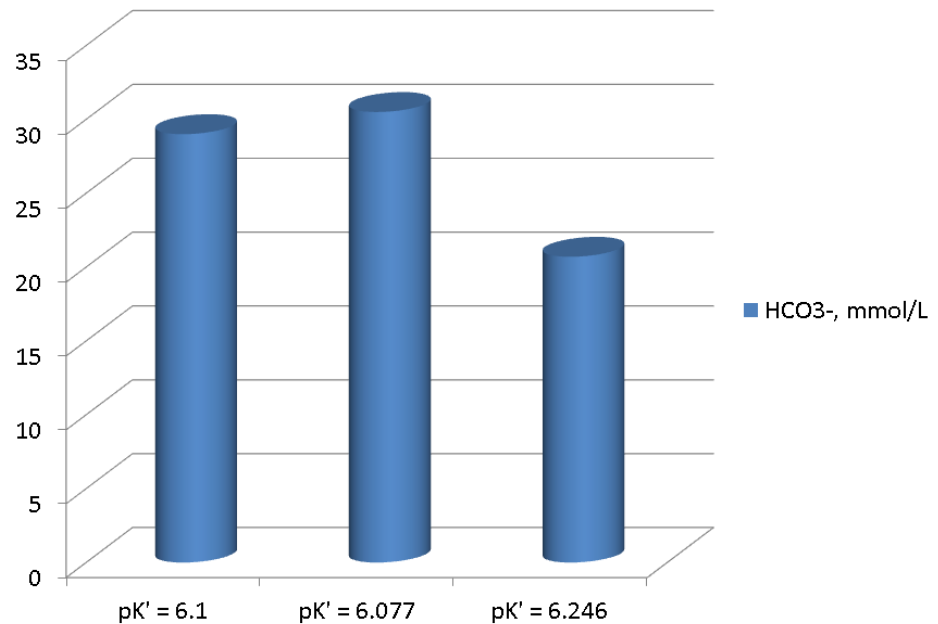


FIGURE 3: Bicarbonate values calculated at a pH of 6.91 from a pCO_2 of 149 mm Hg by various pK' values

High pK' values, observed at low temperatures and low pH values result in low values of calculated HCO_3^- .

The purpose of Figure 3 was to show the direction of the changes in the HCO_3^- concentration that result exclusively from changes in the pK' . Consequently, the pH was kept at 6.91 and the temperature was kept at 37° C in all three calculations. Correction for the effect of temperature on the pCO_2 and use of the correct pK' for each temperature and pH in equation 2 would change the differences shown in this figure and could potentially increase them. Therefore, a variation in the pK' can potentially account for large variations in HCO_3^- and large differences between serum TCO_2 and blood gas HCO_3^- .

We tested the effect of changing pK' on the differences between serum TCO_2 and arterial blood gas HCO_3^- in the patient presented in this report in two sets of calculations. In the first set, we repeated the calculations of HCO_3^- using a pK' of 6.1 by the following equation:

$$HCO_3^- = 0.0301 \times pCO_2 \times 10^{(pH - 6.1)} \quad \{3\}$$

In the second set of calculations, we calculated the approximate pK' value at a pH of 6.91 by performing a linear regression on pH and pK' values at a temperature of 37° C in Table 1-3 of the chapter by Madias and Cohen [1]. We obtained the following regression: $pK' = 6.410 - 0.042xpH$, $r = -0.994$. The pK' values calculated by this regression are 6.120 for a pH of 6.91 and 6.114 for a pH of 7.11. Figure 4 shows HCO_3^- values reported and calculated from equations 3 and 2 for pK' values of 6.1 and 6.120 in the first set of measurements. The calculated values were substantially higher than the reported HCO_3^- value and very close to each other and the measured serum TCO_2 of 29 mmol/L.

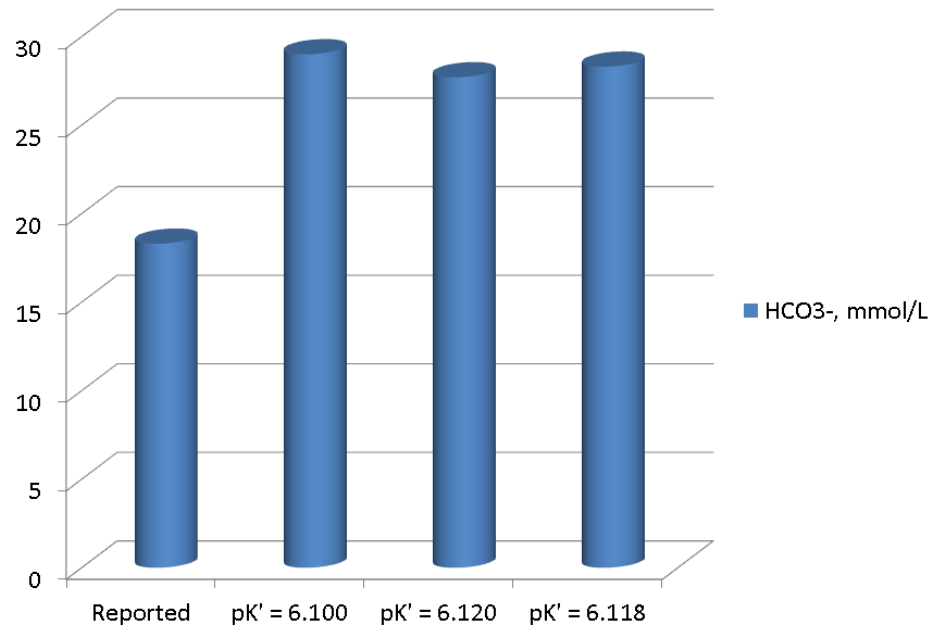


FIGURE 4: Bicarbonate concentrations in the first arterial blood sample reported and calculated by the use of three different pK' values

The three calculated HCO_3^- values were substantially higher than the reported value and very close to each other and to the measured serum TCO_2 .

Figure 5 shows HCO_3^- values reported and calculated from equations 3 and 2 for pK' values of 6.1 and 6.114 in the second set of measurements. Again, the calculated values were substantially higher than the reported HCO_3^- value and very close to each other and the measured serum TCO_2 of 26 mmol/L.

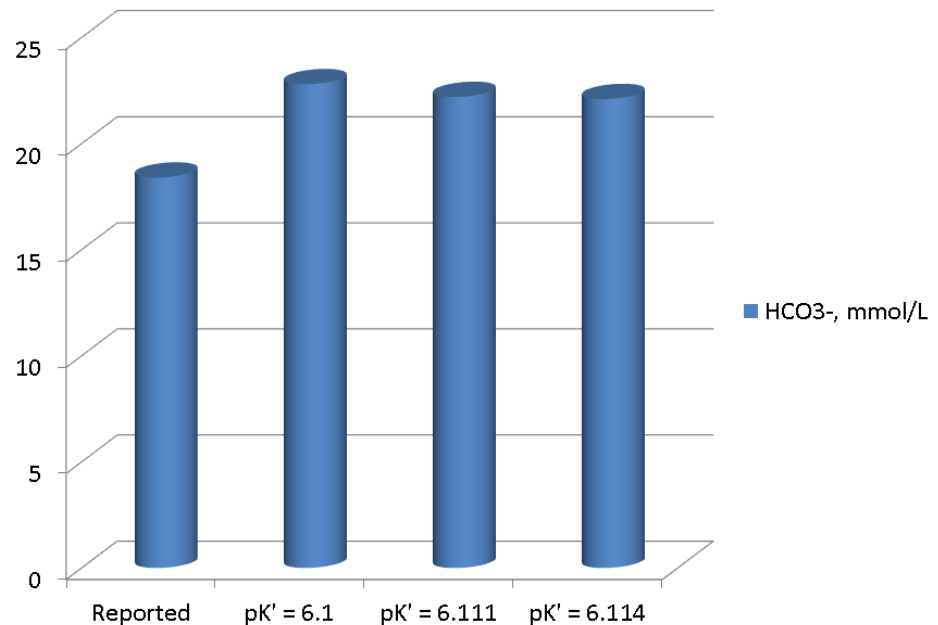


FIGURE 5: Bicarbonate concentrations in the second set of arterial blood gasses reported and calculated by three different pK'.

In the first and second set of blood gasses alike, the calculated HCO₃⁻ values were substantially higher than the reported value and very close to each other and to the corresponding measured serum TCO₂.

The pK' value that is required to obtain an HCO₃⁻ of 18.3 mmol/L, calculated from the Henderson-Hasselbach equation for a pCO₂ of 149 mm Hg and a pH of 6.91 is 6.299, a value substantially higher than any of the values provided in the table published by Madias and Cohen [1]. From equation 1, the pH value required for the calculation of an HCO₃⁻ of 18.3 mmol/L at a pCO₂ of 149 mm Hg is 6.71 when the pK is 6.1. We concluded that it is unlikely that the observed difference between serum TCO₂ and arterial blood HCO₃⁻ was due to variation in the value of pK'.

Potential Effect of Instrument Calibration Error or Human Error

From the previous calculations, we concluded that the reported blood gas HCO₃⁻ values were in error. The similarity of the calculated blood gas HCO₃⁻ values in the first and second sets of measurements suggested a possibility that the error may be in the algorithms of the blood gas apparatus. We investigated whether the error in the calculations of HCO₃⁻ was due to issues related to the blood gas apparatus or to errors of the operators of the blood gas apparatus by

repeating the calculations using the logarithms for the pK' and HCO_3^- calculations shown in the manual of the apparatus. At a temperature of $37^\circ C$, the pK' values calculated by these algorithms are 6.118 for a pH of 6.91 and 6.114 for a pH of 7.11. Figures 4 and 5 show the calculated blood gas HCO_3^- values in the first and second set of measurements by the use of these pK' values. These values are also very close to the other calculated values and the values of the reported serum TCO_2 . We concluded that the error in the reported HCO_3^- values cannot be attributed to the algorithms of the blood gas apparatus.

Discussion

Acid-base disorders indicate health challenges that must be addressed because they result from profound respiratory or metabolic derangements and can be life-threatening. The first step in the management of an acid-base disorder is a correct diagnosis based on accurate measurement or calculation of the acid-base determinants in the blood and on the proper interpretation of these determinants [8]. The diagnosis is based on the observed combination of the measured value of blood pH and the measured or computed values of the pivotal carbonic acid/bicarbonate buffer system [9]. The patient presented in this report illustrates the difficulty encountered when measured and computed acid-base parameters lead to different diagnostic acid-base categories. A pathway leading to deciphering this difficulty is detailed.

In the first two sets of serum and arterial blood gas measurements of our patient, substantial differences were found between the reported concentrations of serum TCO_2 and arterial blood HCO_3^- (Table 1). These differences created differing impressions of the underlying acid-base disorder. The arterial blood gases suggested a picture of mixed respiratory and metabolic acidosis while the combination of arterial pH and pCO_2 , plus serum TCO_2 , indicated acute respiratory acidosis alone [10]. Previous medical history assists in the diagnosis of acid-base disorders [9]. In our patient, previous extensive gut surgery could have been the cause of metabolic acidosis, but his mental status on admission did not allow questioning about recent bowel movements. In any event, the critical issue in this and all other cases with similar findings is an identification of the correct one among the two conflicting acid-base parameters. Variations in the pK' of the carbonic acid/bicarbonate system have been extensively studied as a source of TCO_2/HCO_3^- differences.

Variation of pK' with temperature and acidity of the biological fluid tested was documented in both humans [3-6] and animals [11-12]. It was reported that variation in the pK' values results in substantial variation of the HCO_3^- values calculated by the Henderson-Hasselbach equation [13]. Observed differences between serum TCO_2 and HCO_3^- values calculated by using the Henderson-Hasselbach equation with the pK' fixed at 6.1 were attributed to errors in the value of HCO_3^- secondary to varying pK' [14-20]. This approach suggests that the correct acid-base value for diagnosing the acid-base disorder and assessing its magnitude is the serum TCO_2 , the measurement of which is not affected by changes in pK' .

The concept that the bicarbonate/carbonic acid pK' is the main cause of discrepancies between serum TCO_2 and blood gas HCO_3^- has been challenged [21]. One criticism of this concept is that the scale of the pK' change that has been calculated for changes in blood acidity and temperature is not large enough to explain large differences between TCO_2 and HCO_3^- [22]. As noted, higher values of pK' resulting from high acidity or low temperature of the solution tested lead to lower values of HCO_3^- calculated by the Henderson-Hasselbach equation. Studies

in critically ill patients found only a small variation of the pK' around 6.1 [23-26]. These studies suggest that varying pK' is not the cause of large differences between TCO_2 and HCO_3^- .

Another potential source of differences between serum TCO_2 and blood gas HCO_3^- is an analytical overestimation of TCO_2 [27]. TCO_2 is measured by converting at a temperature of 37° C essentially all CO_2 in the tested sample to HCO_3^- by a complex enzymatic method producing NAD^+ from a known substrate of $NADH$ under the influence of HCO_3^- . The remaining $NADH$ is measured by reflectance spectrophotometry [28]. A large list of tested medications and organic compounds showed no interference with this assay [29]. However, overestimation of TCO_2 was reported in some cases and was attributed to interference by organic acids or carbamino compounds [30-31]. Based on this interpretation of the source of differences between serum TCO_2 and blood gas HCO_3^- , Halperin and coauthors proposed that these differences are not caused by variations in the pK' and that the level of HCO_3^- , rather than that of TCO_2 , is the correct measurement [32]. In a recent case report, serum TCO_2 was consistently low in a patient with normal blood pH and HCO_3^- [33]. The presence in the patient's serum of paraproteins causing turbidity and interfering negatively with the enzymatic measurement of TCO_2 was identified as the source of the erroneously low TCO_2 values in this report.

We evaluated the pK' as the potential source of the difference between serum TCO_2 and blood gas HCO_3^- in the patient presented in this report. The arterial blood pH in the first set of measurements, at 6.91, was lower than the lowest pH value in Table 1-3 of the Madias and Cohen chapter [1]. We calculated the pK' value for a pH of 6.91 by performing a linear regression of pH on the pK' values reported in the same table at a temperature of 37 °C. An apparent linearity of the relationship pK'/pH within "physiologic" pH values has been reported [34]. Changing ionic strength is the source of a curvilinear relationship pK'/pH [7]. However, the ionic strength of the blood of our patient was within normal limits. The slope of the pK'/pH regression that we performed was, at -0.042, within the range of slopes (-0.05 to -0.04) reported in the literature [17]. The pK' values that we calculated were almost identical to the values calculated by the algorithms of the blood gas apparatus. HCO_3^- values calculated using a pK' of 6.1 and pK' values calculated from our pH/ pK' regression analysis of the Madias and Cohen Table 1-3 and from the algorithms of the blood gas apparatus were very close to each other and to the corresponding serum TCO_2 values clarifying the diagnosis of the acid-base disorder and identifying human error as the source of the observed TCO_2/HCO_3^- differences.

Based on the closeness of the HCO_3^- values calculated by using a pK' of 6.1 and pK' values obtained by other methods, we propose that the first step in identifying the source of large TCO_2/HCO_3^- differences should be a recalculation of the blood gas HCO_3^- value using equation 3 with a pK' of 6.1. This calculation is simple and will provide an easy answer to the question of error. If large differences TCO_2/HCO_3^- persist after the first recalculation of the blood gas HCO_3^- , the second step, only in severe hypercapnia, should be to calculate the dCO_2 as $0.0301 \times PCO_2$. Persistence of large TCO_2/HCO_3^- differences should lead to a new calculation of the HCO_3^- using the algorithms of the blood gas apparatus because they may still reveal substantial differences in extreme acid-base disturbances. Persisting differences, unexplained by changes in pK' or pCO_2 , require a systematic investigation of the timing of blood gas and

serum samples in patients with unstable acid-base status and investigation of the handling of the blood samples, with emphasis on the blood gas sample (temperature, time period between drawing and measuring the sample, etc). If sampling issues are discovered, a new simultaneous set of blood gases and serum samples should be obtained. If differences persist in this new set, conditions affecting the measurement of TCO_2 should be investigated [30-31, 33].

Conclusions

Neither a variation in the apparent carbonic acid pK' , nor a high PCO_2 value appears to provide adequate explanations for very large differences between measured serum TCO_2 and calculated blood gas HCO_3^- . Although analytical errors in the measurement of TCO_2 may be present, the possibility of error in the input of values into the blood gas apparatus or in reporting acid-base parameters should be evaluated first. The first, and simple, step in identifying the source of large differences between serum TCO_2 and blood gas HCO_3^- is an independent verification of the calculation of HCO_3^- using a pK' of 6.1. Equation 3 should be used as the algorithm for this calculation.

Additional Information

Disclosures

Human subjects: Consent was obtained by all participants in this study. Raymond G Murphy VA Medical Center IRB issued approval. Approved as a case report with waiving of informed consent with the proviso that all identifying information is removed from the text. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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