REVIEW ARTICLE

Is There a Discordance or Concordance Between Calculated and Measured Plasma/Serum Bicarbonate Concentration?

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Introduction:

In many clinical/medical biochemistry laboratories the urea and electrolyte (U & E) profile includes an assay of plasma/serum bicarbonate concentration ($[HCO_3^-]$). Although the blood gas-calculated $[HCO_3^-]$ usually approximates fairlly closely to the U&E-measured $[HCO_3^-]$ that is not always the case. The extent and cause of discordance between measured and calculated $[HCO_3^-]$ has been the subject of controversy for nearly 30 years.

Many studies addressing this issue have provided conflicting evidence; some have demonstrated clinically acceptable agreement while others have shown quite poor agreement [1,2,3,4]. HCO_3^- is the principal form in which carbon dioxide (CO_2) is transported in blood and is a major blood buffer, helping to maintain the pH of blood surprisingly constant within narrow physiological limits ($[H^+]$: 35 – 45 nmol/L, pH: 7.35 - 7.45). An appreciation of the clinical significance of $[HCO_3^-]$, whether obtained by calculation or assay, depends on a basic understanding of CO_2 transfer and normal acid-base balance [5,6,7]. The present article was therefore designed to answer the question posed in the title itself, being organised into (a) CO_2 transport, (b) acid-base balance, (c) blood gas analysis and calculated $[HCO_3^-]$, (d) U & E profile and measured $[HCO_3^-]$ and (e) concordance or discordance between measured and calculated plasma $[HCO_3^-]$.

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Co_2 transport:

For survival and function, all tissue cells depend on aerobic metabolism to generate energy in the form of adenosine triphosphate (ATP) in the mitochondria with the consumption of oxygen (O_2) and production of CO_2. The ultimate fate of this CO_2 is elimination from the body in expired air, and a fundamentally vital function of blood is transport of CO_2 from tissue cells to the lungs. Due to prevailing higher concentration gradients, CO_2 diffuses out from mitochondria across the cytoplasm and out of the cell into the blood flowing through the capillary network surrounding the cell (Fig.-1). A little of the CO_2 in blood remains dissolved in the water (aquous) phase of blood plasma and an even smaller proportion binds to the amino terminal groups (NH_2) of plasma proteins forming carbamino compounds. Most of the CO_2 diffuses down a concentration gradient into red blood cells (RBCs) where a little

remains dissolved in the RBC cytoplasm, some binds reversibly to NH_2 of haemoglobin (Hb) forming carbamino-Hb, but almost all of the CO_2 entering into RBC is rapidly hydrated to carbonic acid (H_2CO_3) by the red cell isoform of the enzyme carbonic anhydrase (CA). At physiological PH, almost all of this H_2CO_3 rapidly dissociates into HCO_3^- and hydrogen ions (H^+). The H^+ are buffered by

The CO_2 concentration gradient across the alveolar membrane determines that dissolved CO_2 passes from blood to alveoli when blood reaches the lungs (Fig.-2). This loss of CO_2 from blood favours reversal of the red cell reactions described previously (Fig.-1) and HCO_3^- passes from plasma into the RBCs, buffering H^+ released from Hb as it becomes oxygenated. CO_2 produced by reversal of the

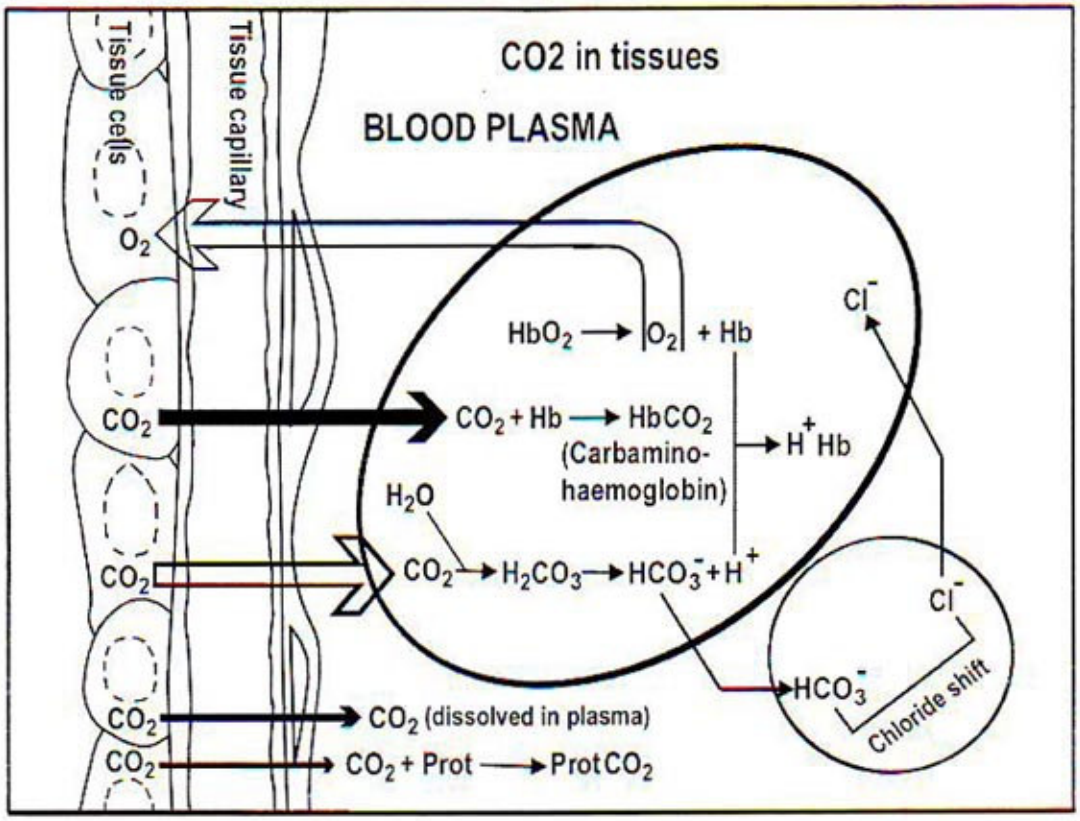

Figure-1: Carbon dioxide in the tissues.

reduced Hb and CO_2 released goes to the CO_2 pool inside the RBCs and most of the HCO_3^- passes from RBCs to plasma in exchange for chloride ions (Cl^-) (known as chloride shift) maintaining electrochemical neutrality[5,6,7].

CA reaction diffuses down the concentration gradient from RBCs to plasma and onwards to the alveoli [5,6,7].

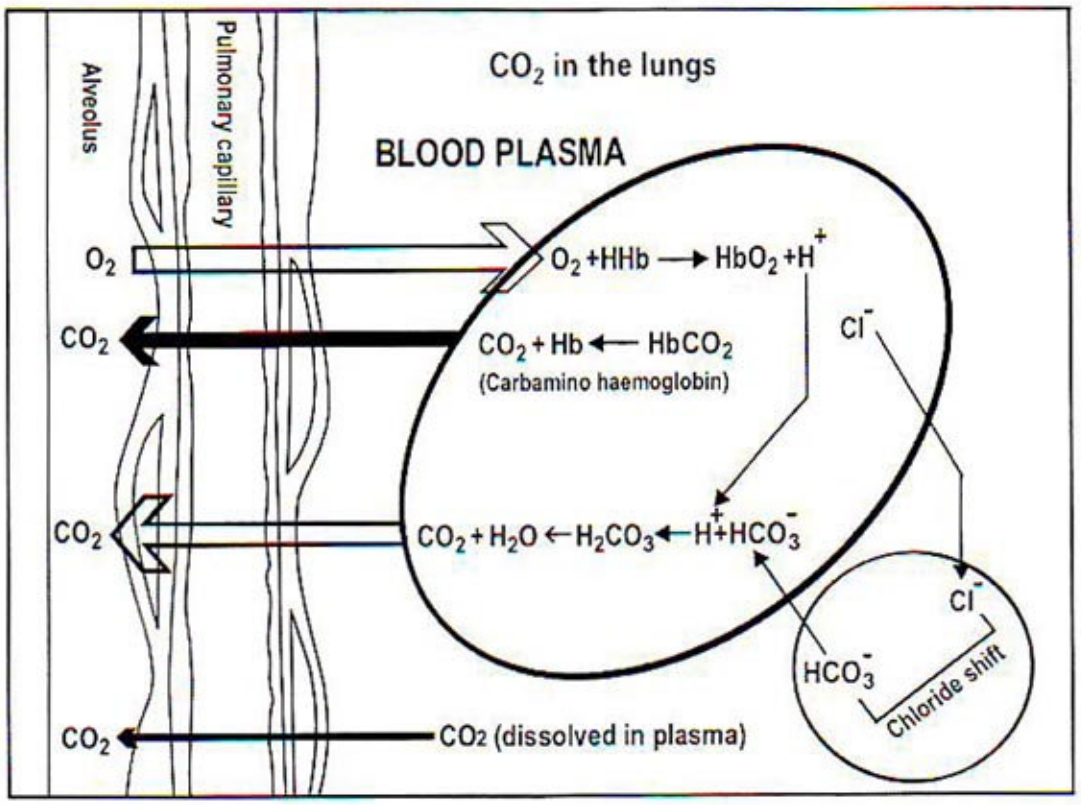

Figure-2: Carbon dioxide in the lungs.

Mixed venous blood arriving at the lungs has an approximate total CO_2 content of 23.5 mmol/L, whereas arterial blood leaving the lungs has a total CO_2 content of 21.5 mmol/L. This arteriovenous difference (2.0 mmol/L) represents the CO_2 added to blood from tissue cells and lost from blood to be eventually excreted from the body in expired air. It should therefore be clear from the above that though most of CO_2 is transported in blood plasma as HCO_3^-, there are in total four modes of transport: (i) 95% is transported as HCO_3^-, (ii) about 50% is transported as CO_2 simply dissolved in blood plasma, (iii) less than 1.0% is transported bound to plasma proteins (carbamino compounds), (iv) less than 0.01% is transported as H_2CO_3 [4,5,6].

Acid-base balance:

The major determinant of blood H^+/pH is the CO_2 in blood due to its hydration reaction to H_2CO_3 (Table-I). As [CO_2] rises, [H^+] rises too in the blood. Therefore, regulation of blood [CO_2], i.e. matching the rate of CO_2 production in the mitochondria of tissue cells to the rate of CO_2 elimination through the respiratory system, is vital for maintaining normal blood pH. Respiratory rate, controlled by CO_2, pCO_2-sensitive chemoreceptors located in the brain stem and carotid artery, is increased if pCO_2 rises and decreased if pCO_2 declines. Decreased respiratory rate promotes CO_2 retention and increased respiratory rate promotes CO_2 elimination. The relationship between blood pH and CO_2 is described by a form of the Henderson – Hasselbach equation

(Table-I, Equation-2), which is derived from application of the law of mass action to the hydration and dissociation reactions shown in Equation-1. Blood pH is dependent on the ratio of plasma [HCO_3^-] (metabolic component) to pCO_2 (respiratory component). If pCO_2 falls/decreases without an equivalent decrease/fall in [HCO_3^-], pH rises and conversely, if pCO_2 increases/rises without equivalent rise/increase in [HCO_3^-], pH falls/decreases. For HCO_3, decreased [HCO_3^-] without equivalent fall in pCO_2 results in decreased pH [5,6,7].

Blood gas analysis and calculated [HCO_3^-]:

The blood gas analysers generate the following CO_2 parameters: (i) partial pressure of CO_2, i.e. pCO_2 (kPa), (ii) plasma HCO_3^- (mmol/L) and (iii) H^+/pH. The measurement is made using CO_2-specific pH electrode incorporated in blood gas analysers. In normal condition of health, pCO_2 of arterial blood is maintained within the range of 4.7 – 6.0 kPa, while pCO_2 of venous blood is little higher at 5.6 – 6.8 kPa. pCO_2 is a measure of the pressure exerted by that small amount (≡ 5.0%) of total CO_2 (TCO_2) in blood that remains in the gaseous state 'dissolved in' the aqueous phase of plasma i.e., dissolved CO_2 [dCO_2]. [HCO_3^-] is the form in which most CO_2 (≡ 95%) is transported in plasma. It cannot be measured but is obtained during blood gas analysis by calculation using Equation-4 which is a rearrangement of Equation-2 (Table-I) and dependent on pH and pCO_2, both measured during blood gas analysis. In normal condition of health, arterial and venous plasma [HCO_3^-] are maintained within the reference range of 22–28 mmol/L and 24–30 mmol/L approximately. For all practical purposes, TCO_2 in blood can be considered as the sum of [HCO_3^-] and dCO_2 because H_2CO_3 and carbamino compounds are present in very small concentration [5,6,7].

Table-I: The equations and the Henderson-Hasselbach equation

EQUATION- 1	$CO_2 + H_2O \xleftrightarrow{\text{carbonic anhydrase}} H_2CO_3 \longleftrightarrow HCO_3^- + H^+$
EQUATION- 2	$pH = pK_1 + \log \frac{[HCO_3^-]}{S \times pCO_2}$ where: pK_1 = 'apparent' dissociation constant of carbonic acid = 6.1; [HCO_3^-] = concentration of plasma bicarbonate (mmol/L); S = solubility coefficient for CO_2 at 37^0C (0.23); pCO_2 = partial pressure of CO_2 (kPa); The denominator in this equation (S x pCO_2) is the concentration of carbon dioxide (mmol/L) dissolved in blood plasma (i.e. around 5% of total CO_2).
EQUATION- 3	By removing all constants, Equation-2 can be simplified to: pH α [HCO_3^-]/pCO_2
EQUATION- 4	$\log [HCO_3^-] = pH + \log (S \times pCO_2) - pK_1$

Urea and electrolyte profile and measured [HCO_3^-] :

The traditional urea and electrolyte U & E profile of blood plasma includes [HCO_3^-] measurement. As U & E is requested much more frequently than arterial blood gases, measured [HCO_3^-] can provide the first indication of disturbance in CO_2 (and therefore acid-base) homeostasis. The use of the term HCO_3^- to describe this parameter is not strictly accurate because the chemical methods measure all CO_2 liberated from plasma (or serum) by the addition of strong acid, or alternatively all HCO_3^- produced as the result of adding a strong alkali to plasma (or serum). Thus, measured [HCO_3^-] includes not only HCO_3^- but also dCO_2, carbamino compounds and H_2CO_3 Therefore, "total CO_2, i.e. [TCO_2]" is the more correct term used by some laboratories to describe measured [HCO_3^-] [5,6,7].

Measured [HCO_3^-] and calculated [HCO_3^-] discordance or concordance?

The two genuine theoretical reasons for measured [HCO_3^-] to be slightly higher than calculated [HCO_3^-] are: (i) Firstly, measured [HCO_3^-] includes an additional component of approximately 1.2 mmol/L as non-bicarbonate CO_2; (ii) Second, venous blood plasma or serum used to measure [HCO_3^-] has a slightly higher [HCO_3^-] than arterial blood plasma or serum used for calculated [HCO_3^-]. In general, notwithstanding these theoretical differences which amount not more than 2.0 mmol/L, calculated and measured [HCO_3^-] do agree in general but not always the case. Despite numerous studies over the years, however, there remains no consensus on the cause or extent of the non-systematic discordance [1,2,3,4]. In recent years, several studies have identified a particular patient group, i.e. critically ill patients, as more likely to show discordance as high as 15 mmol/L. This has led to strong suggestion that for critically ill patients at least it might be wise to abandon calculated [HCO_3^-] in favour of measured [HCO_3^-]. In addition, it was suggested that the more easily available serum [HCO_3^-] could approximate base deficit (BD) and potentially serve as a useful endpoint of resuscitation (EOR) in critically ill patients [8,9,10]. A most recent study comparing measured and calculated [HCO_3^-] was published from the Mayo Clinic in Rochester, Minnesota, USA. Investigators retrieved 17,621 computer record of both measured and calculated [HCO_3^-] for samples drawn simultaneously from the same patient over a 10 month period spanning 2006–2007. The calculated values were obtained using a Radiometer 725 blood gas analyser and the assay for measured values was an enzymatic (phosphoenolpyruvate) method performed on a Roche discrete analyser and the [HCO_3^-] range obtained was 5.0 – 49.0 mmol/L. Statistical analyses revealed the regression equation for measured (y) and calculated (x) [HCO_3^-] as the following : $y=0.96x+0.68$ mmol/L. The mean (standard deviation [SD]) difference between measured and calculated [HCO_3^-] was just – 0.36 (1.23) mmol/L. Values agreed to within 2.0 mmol/L of each other for 16,800 pairs (95.3%) and within 3.0 mmol/L for 17,538 pairs (98.3%) of 17,621 pairs. Just about 0.65% of paired results demonstrated what might be considered as clinically significant discordance (difference > 4.0 mmol/L). This very large study has provided reliable evidence that for the vast majority of patients it is immaterial whether [HCO_3^-] is determined by calculation or

biochemical assay [11,12]. However, small percentage of paired results demonstrating discordance can not be ignored totally.

In conclusion, the extent and cause of discordance between measured and calculated [HCO_3^-] has been the subject of controversy for many years. A most recent large database study produced reliable evidence that for the vast majority of the patients it is immaterial whether [HCO_3^-] is determined by calculation or biochemical assay. The small percentage of paired results demonstrating discordance, however, can not be ignored totally. Several studies done in recent years have identified a patient group, i.e. the critically ill patients, as more likely to show discordance, and advised that for critically ill patients at least, it might be prudent to abandon calculated [HCO_3^-] in favour of measured [HCO_3^-]. As arterial puncture is painful, invasive and costly, it was suggested that the more easily available serum [HCO_3^-] may be safely and accurately substituted for arterial BD measurement in critically ill patients. A prospective study comparing serum [HCO_3^-] to BD could confirm serum [HCO_3^-] as a useful marker for EOR in critically ill patients.

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