Quantitative Accuracy of the Simplified Strong Ion Equation to Predict Serum pH in Dogs

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N.J. Cave and S.T. Koo

Background: Electrochemical approach to the assessment of acid-base states should provide a better mechanistic explanation of the metabolic component than methods that consider only pH and carbon dioxide.

Hypothesis/Objectives: Simplified strong ion equation (SSIE), using published dog-specific values, would predict the measured serum pH of diseased dogs.

Animals: Ten dogs, hospitalized for various reasons.

Methods: Prospective study of a convenience sample of a consecutive series of dogs admitted to the Massey University Veterinary Teaching Hospital (MUVTH), from which serum biochemistry and blood gas analyses were performed at the same time. Serum pH was calculated (H_{cal}^+) using the SSIE, and published values for the concentration and dissociation constant for the nonvolatile weak acids (A_{tot} and K_a), and subsequently H_{cal}^+ was compared with the dog's actual pH ($H_{measured}^+$). To determine the source of discordance between H_{cal}^+ and $H_{measured}^+$, the calculations were repeated using a series of substituted values for A_{tot} and K_a.

Results: The H_{cal}^+ did not approximate the $H_{measured}^+$ for any dog ($P = 0.499$, $r^2 = 0.068$), and was consistently more basic. Substituted values A_{tot} and K_a did not significantly improve the accuracy ($r^2 = 0.169$ to <0.001). Substituting the effective SID ($A_{tot} - [HCO_3^-]$) produced a strong association between H_{cal}^+ and $H_{measured}^+$ ($r^2 = 0.977$).

Conclusions and clinical importance: Using the simplified strong ion equation and the published values for A_{tot} and K_a does not appear to provide a quantitative explanation for the acid-base status of dogs. Efficacy of substituting the effective SID in the simplified strong ion equation suggests the error lies in calculating the SID.

Key words: Acid-base; Canine; Electrochemical; Strong ion difference.

In 1978, Peter Stewart introduced a new method for understanding acid-base regulation, which he termed the "electrochemical approach".[1] Stewart argued that only the variables that independently affect the hydrogen ion concentration should be considered, and identified them as the partial pressure of CO_2 (PCO_2), the strong ion difference (SID), and the concentration of nonvolatile weak acids (A_{tot}). This method contrasted with the established approach of only considering the relationship between the pH, PCO_2, and HCO_3^- as described by Henderson.[2] A criticism of Henderson's equation is that it does not assist in identifying the nature of the metabolic component of an acid-base disturbance. In addition, bicarbonate is not directly measured but is derived from the measured pH and PCO_2 and thus gives no information independent of those values. In contrast, the electrochemical approach should provide a better mechanistic explanation of the metabolic

component of an acid-base disturbance, and should provide a more complete conception of the variables that determine an animal's plasma pH.

The electrochemical approach requires measurement of PCO_2, calculation of the SID from serum electrolytes and lactate, quantification of the electrochemical charge contributed by weak acids (A_{tot}), and knowledge of the overall dissociation constant for the nonvolatile weak acids (K_a). While PCO_2 is easily measured and the SID is simply calculated from a standard serum biochemistry analysis, the values for A_{tot} and K_a are species specific, and are not easily determined. Albumin is the single largest contributor to A_{tot} in a healthy animal, while other plasma proteins and phosphate contribute less. Constable and Stampfli[3] determined the association between A_{tot} and serum albumin concentration in dogs, and calculated the K_a for canine plasma, using 10 normal dogs. As the authors stated, applying those values should improve our understanding of the mechanism for acid-base disturbances in dogs.

The clinical utility of either approach to understanding acid-base status should be judged ultimately by its efficacy in guiding treatment or prognostication. The standard and the electrochemical approaches have been compared in human patients to determine if clinical outcome is improved using the different approaches to assessing patient acid-base status. Some authors have claimed superiority for the electrochemical approach,

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while others did not find any clinically relevant advantage.[4,5] Another method to judge the utility of the electrochemical approach is to quantitatively validate the calculations. If the variables included in the electrochemical approach truly determine pH, then the variables can be used to calculate the patient's actual measured pH. A valid and complete mathematical expression will yield a calculated pH that equals the

$$H_{cal}^+ = \frac{[K_1' \times SCO_2 \times PCO_2] + [K_a \times A_{tot}] - [K_a \times SID] + \sqrt{((K_1' \times SCO_2 \times PCO_2 + [K_a \times SID] + [K_a \times A_{tot}])^2 - 4 \times K_a^2 \times SID \times A_{tot})}}{2[SID]}$$

measured pH.

This study was conducted to test the hypothesis that the electrochemical approach, using the published canine values for K_a and A_{tot}, can be used to calculate the pH of serum from dogs. If it could, we would conclude that the approach correctly utilizes the independent variables that determine pH, and hence truly explains a patient's acid-base status.

Materials and Methods

Study Population

This study comprised a convenience sample of a consecutive series of dogs admitted to the intensive care unit of the Massey University Veterinary Teaching Hospital (MUVTH), from which serum biochemistry and blood gas analyses were performed at the same time. No other inclusion or exclusion criteria were applied.

Blood Collection

Two samples of venous blood were drawn from dogs admitted to the ICU at the MUVTH. The first sample was collected anaerobically into a 1-mL polypropylene syringe. Heparin (DBL Heparin Sodium injection, 5,000 iU/mL) was added to the syringe before collection by drawing up 250 µL of 0.9% saline, to which heparin had been added to give a concentration of 10 iU/mL. The heparin saline solution was then evacuated through the needle, leaving only the volume held within the needle and syringe hub. Blood was collected using the same needle. The sample was immediately analyzed with a blood gas analyzer (i-STAT Portable Analyzer 108952, with i-STAT CG4 + cartridges, Abaxis Inc, Union City, CA). The second sample of venous blood was collected using a plain syringe, and placed into plain serum tubes for biochemistry analysis. The serum biochemistry analysis was completed within 2 hours of collection at a commercial laboratory.[a] using an automated commercial analyzer.[b]

Calculation of Strong Ion Difference and the Strong Ion Gap

The SID (mEq/L) for each patient was defined as $([Na^+] + [K^+]) - ([Cl^-] + [Lactate^-])$ and was matched with the appropriate value for A_{tot} as described by Constable and Stampfli.[3] The strong ion gap (SIG, mEq/L) was calculated using 2 different methods as reported by Fettig et al. (2012).[6] SIG_1 was defined as $= [Na^+] + [K^+] - [Cl^-] - [Lactate^-] - A_{tot} - [HCO_3^-]$.

SIG_2 was defined as [albumin (g/L)] $\times$ 0.49 $-$ anion gap.[3] All analytes were expressed as mmol/L.

Calculation of H_{cal}^+ and $H_{measured}^+$

Strictly speaking, the concentration of hydrogen ions is not measured, but it is the hydrogen ion activity that is represented by the term "H^+". The $H_{measured}^+$ was defined as the inverse logarithm of the negative value of the measured pH, assuming an activity coefficient of 1. The H_{cal}^+ was calculated by the following simplified strong ion equation,[7] using the values published by Constable and Stampfli.[3]

where A_{tot} (mmol/L) = [total protein] $\times$ 0.272;

$K_a = 4.17 \times 10^{-4}$;

$K_1' = 7.5857 \times 10^{-4}$;

and SCO_2 (mmol L^{-1} mmHg^{-1}) = 0.0307

Determination of the Accuracy of the Simplified Strong Ion Equation for Calculating H_{cal}^+

The correlation between the H_{cal}^+ and $H_{measured}^+$ was assessed by linear regression analysis.[c] Because of the discrepancy between the H_{cal}^+ and $H_{measured}^+$, we varied our input to the simplified strong ion equation in an attempt to determine (a) where the source of error might be, and (b) the magnitude of any error in a particular variable needed to generate the discrepancy. We assumed the correctness of the published values for K_1' and SCO_2, and the accuracy of the measured pH and PCO_2. Thus, we adjusted the 3 remaining variables:

1.   SID. In an attempt to incorporate any significant unmeasured anions, the SID was "corrected" for the SIG, by subtracting SIG_1 and SIG_2 separately from the SID. An attempt to correct the SID was also made, by correcting for the net strong ion charge attributed to total protein and phosphate as reported by Constable and Stampfli.[3] This equated to 0.184 mEq/g total protein, and 1 mEq/L for phosphate.

2. K_a. With the other variables kept constant, we applied the different published values of K_a in canine plasma, namely 0.17×10^{-4}, 4.84×10^{-4}, and 7.83×10^{-4}. These values were derived by Constable and Stampfli[3] using isolated canine plasma and 3 different SID formulae.

3. A_{tot}. With the other variables kept constant, we replaced the calculated A_{tot} with fixed values of 15 mmol/L, 25 mmol/L, and 35 mmol/L.

The correlation between the H_{cal}^+ and $H_{measured}^+$ using different values of K_a and A_{tot} was assessed by linear regression analysis.

Results

Patients

A total of 10 dogs were included in the study. The dogs were terrier cross breeds (n = 4), New Zealand Huntaways (n = 2), an Irish Wolfhound, a Fox Terrier, a Labrador, and a Border Collie. The median age was

Table 1. Mean and range of biochemistry values of the blood samples collected from 10 dogs with various conditions

Variable	Mean	Range	Reference Range
pH	7.37	7.23–7.48	7.408 (0.03)[a]
PCO_2 (mmHg)	30.5	21.7–41.6	36.4 (2.97)[a]
Na^+ (mmol/L)	147.9	142.0–153.0	139–153[b]
K^+ (mmol/L)	4.2	3.6–5.1	3.5–5.6[b]
Ca^{2+} (mmol/L)	2.52	2.29–2.86	2.2–3.0[b]
Cl^- (mmol/L)	113	103.0–125.0	105–121[b]
$Lactate^-$ (mmol/L)	1.3	0.3–5.0	
HCO_3^- (mmol/L	17.7	11.4–23.9	23 (1.5)[a]
Total protein (g/L)	58.3	42–69	52–75[b]
Albumin (g/L)	33.6	24–44	26–44[b]
Globulin (g/L)	24.8	17–36	17–39[b]
Phosphate (mmol/L)	1.35	0.73–2.96	1.0–3.0[b]
SID (mEq/L)	37.8	29.9–45.0	40.7 (4.6)[c]
SIG_1 (mEq/L)	6.7	1.4–16.7	7.1 (2.3)[d]
Corrected SID (mEq/L)	31.0	21.2–40.0	

[a]Mean (SD) values of 13 beagles using the i-STAT analyser.[9]
[b]Laboratory range.[a]
[c]Mean (SD) from 58 healthy dogs.[10]
[d]Reference intervals from 54 healthy dogs.[8]

8.5 years (range 4 to 14 years) and the dogs were diagnosed with neoplasia (n = 3), acute kidney disease (n = 2), polyradiculoneuritis, seizures, polymyopathy, pulmonary fibrosis, and fever of unknown origin.

Blood Analysis and Calculation of SID, SIG_1, and SIG_2

The biochemical values for the blood samples collected from the study population are presented in Table 1.

Accuracy of Simplified Strong Ion Equation to Predict $H^+_{measured}$

The H^+_{cal} did not approximate the $H^+_{measured}$ for any dog, and was consistently more basic. The correlation between H^+_{cal} and $H^+_{measured}$ is depicted in Fig 1. Next, we determined if the accuracy of the simplified strong ion equation could be improved if different values of pK_a and A_{tot} were used. When tested by linear regression using the 6 different values for K_a and A_{tot}, the correlation between H^+_{cal} and $H^+_{measured}$ ranged from $r^2 = 0.0244$ when K_a was equal to 7.83×10^{-4} ($P = 0.048$), to $r^2 < 0.001$ when A_{tot} was equal to 25 mmol/L ($P = 0.966$). Correcting the SID for the net strong ion charge of total protein and phosphate did not improve the correlation ($r^2 = 0.142$, $P = 0.283$). When the SID was corrected for the SIG using SIG_2, the correlation between H^+_{cal} and $H^+_{measured}$ was not improved ($r^2 = 0.169$, $P = 0.17$). However, when the SID was corrected for the SIG using SIG_1, the correlation between H^+_{cal} and $H^+_{measured}$ was very strong (Fig 2, $r^2 = 0.977$, $P < 0.001$).

Discussion

Using the electrochemical approach to interpret the acid-base status of a patient should yield a better mech-
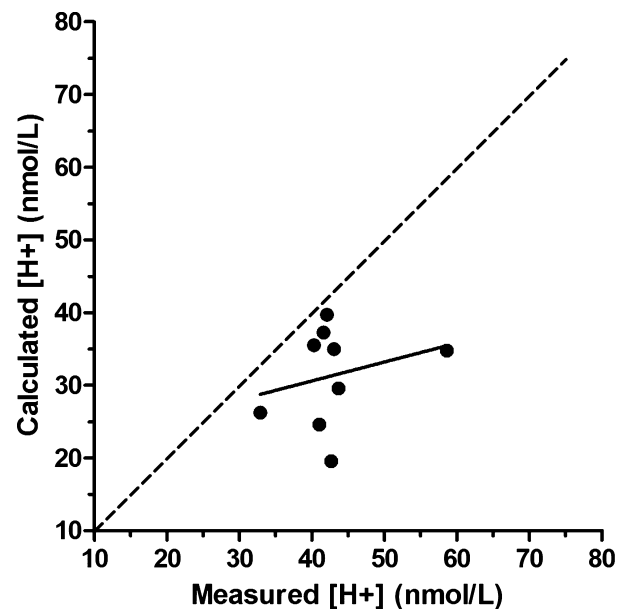

Fig 1. The association between the measured concentration of H^+ and the concentration of H^+ calculated using the simplified strong ion equation and the published values of A_{tot} and K_a in 10 dogs.[3] There was no significant association ($P = 0.4985$, $r^2 = 0.0678$). Solid line = regression line, dashed line = line of identity.

anistic explanation of the metabolic component of an acid-base disturbance, and should provide a more complete understanding than Henderson's approach. The qualitative utility of the approach has been evaluated in dogs, and calculating the strong ion gap has been qualitatively useful in detecting the presence of organic anions not routinely measured ("unmeasured anions").[8] However, if the electrochemical approach is quantitatively useful in clinical settings, it should be able to predict the patient pH from the measured biochemical variables governing the pH.
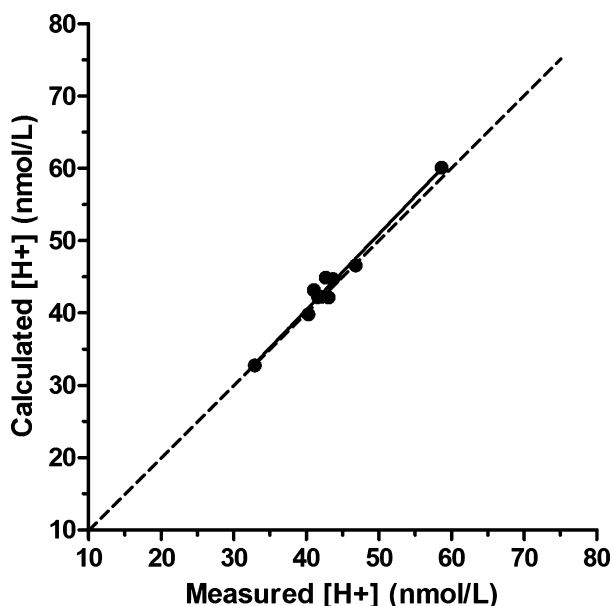
Fig 2. The association between the measured concentration of H^+ and the calculated concentration of H^+ in 10 dogs ($P < 0.001$; $r^2 = 0.9767$). The H^+ was calculated using the simplified strong ion equation, published values of A_{tot} and K_a, and the effective strong ion difference $(A_{tot} + [HCO_3^-])$ was substituted for the calculated SID.[3] Solid line = regression line, dashed line = line of identity.

In this study, we evaluated the accuracy of the electrochemical approach to predict the patient pH using the previously published values for K_a and A_{tot}, and we found no significant correlation between the $H^+_{measured}$ and H^+_{cal}, and that the H^+_{cal} did not approximate the $H^+_{measured}$ for any dog.[3] We then tested the approach using different fixed values for A_{tot}, K_a, and also allowing for inaccuracy in measurement of the variables contributing to the SID, and yet the H^+_{cal} did not even approximate the $H^+_{measured}$. It is not suggested that the different fixed values for A_{tot} and K_a could have been correct, or even physiologically plausible, but the exercise was conducted to determine if the inaccuracy between H^+_{cal} and $H^+_{measured}$ could be resolved by even massive adjustment of those individual terms.

The possible reasons for such discrepancies between H^+_{cal} and $H^+_{measured}$ include: (1) inaccuracy of the constants used in the equation, (2) inaccuracies in the measurements of 1 or more analytes, (3) the presence of unmeasured strong anions, (4) an intrinsic error in the calculations of the H^+_{cal}, and (5) inaccuracy of the underlying theory, the SID equation, or both.

We believe that the constants cannot be the sole reason for the discrepancies because the error in the H^+_{cal} is so large and inconsistent, and there was only a weak association between H^+_{cal} and $H^+_{measured}$ in the 10 dogs. Nonetheless, the fact that $H^+_{measured}$ is always greater than H^+_{cal} suggests there is at least a component of a systematic error. We did not control for temperature before the analysis of our sample, but the effect size is unlikely to explain the error. However, the specific enthalpy change for the dissociation of canine albumin and other contributors to total weak acids have not been determined, and therefore we are unable to ascertain the degree of influence of temperature to H^+_{cal}.

Inaccuracies in the measurements of 1 or more analytes would be a plausible reason for the random error. It might be that the sum of errors from each analyte creates a large enough random error to explain the discordance. The variable in the strong ion equation that is most likely to suffer from accumulated error is the SID. This is consistent with a recent study of the electrochemical approach in diseased horses, in which it was shown that analysis of electrolytes using indirect potentiometry and sample dilution is insufficiently accurate to apply the simplified strong ion equation.[9] In addition, the electrochemical approach assumes that the majority of anion activity in plasma is accounted for by anions that are routinely measured. The presence of unmeasured strong anions or weak acids results in H^+_{cal} less than the $H^+_{measured}$. Unmeasured anions are indirectly detected by calculating the strong ion gap (SIG). The molecular moieties that account for the majority of the increase in unmeasured anion activity in dogs with experimental hemorrhagic shock have been identified.[8] In that study, the strong ion gap rose to 5.1 ± 2.2 mEq/L. We found that correcting the SID for apparent unmeasured anions by subtracting the calculated SIG_1 value from the SID, produced an almost perfect correlation. Although this appears satisfactory, correcting the SID in this manner simply equates to what is termed the effective SID (SID_e), and is equal to $(A_{tot} + [HCO_3^-])$. The SID_e does not include any calculation involving electrolytes, includes the concentration of HCO_3^- which is derived using the measured pH, and cannot be used as a substitute for SID in the SSIE. Thus, the calculated SIG_1 cannot be used to produce a corrected SID, which simply reflects the relationship between pH and the derived concentration of HCO_3^-, with a minor modification from the effect of A_{tot}. However, this finding suggests that the error is in the calculation of the SID, and that although the values we used for A_{tot} and K_a appear to be accurate despite being derived using the same SID calculation, the SID calculation needs to be different for clinically abnormal patients.

The electrochemical approach to the understanding of acid–base balance has the potential to provide the most complete qualitative explanation of acid–base balance in the body. We believe that a quantitative approach can be judged by the accuracy it predicts a patient's pH. When applied to the results from the analysis of the dogs in this study, the electrochemical approach did not have quantitative utility unless the SID_e was substituted. The explanation for this remains obscure, though poor accuracy of electrolyte measurement seems most likely.[10,11]

Footnotes

Acknowledgments

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