Use of Sodium-Chloride Difference and Corrected Anion Gap as Surrogates of Stewart Variables in Critically Ill Patients

Jihad Mallat*, Stéphanie Barrailler, Malcolm Lemyze, Florent Pepy, Gaëlle Gasan, Laurent Tronchon, Didier Thevenin

Department of Intensive Care Unit, Centre Hospitalier du Dr. Schaffner, Lens, France

Abstract

Introduction: To investigate whether the difference between sodium and chloride ($[Na^+] - [Cl^-]$) and anion gap corrected for albumin and lactate (AG_{corr}) could be used as apparent strong ion difference (SID_{app}) and strong ion gap (SIG) surrogates (respectively) in critically ill patients.

Methods: A total of 341 patients were prospectively observed; 161 were allocated to the modeling group, and 180 to the validation group. Simple regression analysis was used to construct a mathematical model between SID_{app} and $[Na^+] - [Cl^-]$ and between SIG and AG_{corr} in the modeling group. Area under the receiver operating characteristic (ROC) curve was also measured. The mathematical models were tested in the validation group.

Results: in the modeling group, SID_{app} and SIG were well predicted by $[Na^+] - [Cl^-]$ and AG_{corr} ($R^2 = 0.973$ and 0.96, respectively). Accuracy values of $[Na^+] - [Cl^-]$ for the identification of SID_{app} acidosis (<42.7 mEq/L) and alkalosis (>47.5 mEq/L) were 0.992 (95% confidence interval [CI], 0.963–1) and 0.998 (95%CI, 0.972–1), respectively. The accuracy of AG_{corr} in revealing SIG acidosis (>8 mEq/L) was 0.974 (95%CI: 0.936–0.993). These results were validated by showing excellent correlations and good agreements between predicted and measured SID_{app} and between predicted and measured SIG in the validation group ($R^2 = 0.977$; bias = 0±1.5 mEq/L and $R^2 = 0.96$; bias = −0.2±1.8 mEq/L, respectively).

Conclusions: SID_{app} and SIG can be substituted by $[Na^+] - [Cl^-]$ and by AG_{corr} respectively in the diagnosis and management of acid-base disorders in critically ill patients.

Introduction

Disorders of acid-base balance are among the most common abnormalities seen in critically ill patients [1]. They are generally related to clinical outcomes and disease severity, especially for metabolic acidosis [1,2]. Acid-base disorders are currently assessed by three different methods: the physiological approach [3,4], the base excess approach [5,6], and the physicochemical approach [7,8]. The first two approaches, which are based on the analysis of plasma concentration of bicarbonate and standard base excess (SBE), and further completed by the use of plasma anion gap (AG) are the most widely methods used to evaluate the metabolic component of acid-base disturbances [9]. One advantage of these methods is that are easy to understand and apply in common clinical situations [9]. However, the SBE is a calculated figure derived from $PaCO_2$ and arterial pH, but reliance on its use alone to quantify metabolic disturbances has a number of pitfalls. First, it cannot identify whether an acidosis is due to increased tissue acids, hyperchloremia, or a combination of both. Second, its calculation assumes normal plasma protein, which may limit its accuracy in

the critically ill patients [10,11]. On the other hand, AG is grossly underestimated in the presence of hypoalbuminemia, which is a frequent occurrence in critically ill patients [12].

An alternative evaluation is the mathematical model based on physiochemical principles described by Stewart [8], and modified by Figge [11,13]. This theory states that three independent variables determine pH in plasma by changing the degree of water dissociation into hydrogen and hydroxide ions. The three independent variables are $PaCO_2$; strong ion difference (SID), which is the difference between fully dissociated plasma anions and cations; and plasma weak acids, namely, albumin and phosphate. This method allows the clinician to quantify individual components of acid-base abnormalities and provides insight into their pathogenesis [14]. Many studies showed that this approach, compared to the traditional approaches, is the best to identify acid-base disorders in the population of critically ill patients [15,16]. Nevertheless, the Stewart's approach is a time-consuming method and unsuitable at the bedside. Previous studies [16,17] have shown that strong ion gap (SIG) could be substituted by the anion gap corrected for albumin and lactate (AG_{corr}). Furthermore, recently,

Nagaoka et al [18] suggested that the difference between sodium and chloride concentrations ($[Na^+] - [Cl^-]$) could be used as a surrogate of SID. Nevertheless, in these studies there was no independent sample of patients to validate these findings. Furthermore, the temporal evolution of these surrogates was not tested. Therefore, the aim of our study was to investigate whether AG_{corr} and $[Na^+] - [Cl^-]$ difference could be used as SIG and SID surrogates respectively in critically ill patients, and to validate these results in a different sample of patients.

Methods

Ethics Statement

This study was approved by the Institutional Ethics Committee (comité d'éthique du centre hospitalier du Dr. Shaffner de Lens). As the blood tests and data collected in this study were all standard clinical practice, the requirement for informed written consent was waived, and only oral consent was obtained. There were no measures taken to document to verbal consent procedure; nevertheless, the entire consent procedure was submitted to the ethics committee before they approved this study. If the patient or his/her next of kin refused consent, patient's data were not entered into analysis.

Clinical and laboratory data

A total of 341 patients admitted to the intensive care unit (ICU) of a general hospital from February to December 2011 were enrolled in the study. All data were retrieved from a prospectively collected database.

Clinical and laboratory data were collected from all patients at admission, and laboratory data were recorded again 24 hours later only in the sample of patients who served as a cross-validation group. The following clinical data were recorded: age, sex, Simplified Acute Physiology Score (SAPS II), cause of ICU admission, length of stay, and outcome. All arterial samples were analyzed in the central laboratory of the institution (Cobas 6000; Roche Diagnostics, Meylan, France). Na^+, K^+, and Cl^- were measured using the ion-selective electrode technique. Magnesium and phosphate concentrations were determined by colorimetric techniques (chlorophosphonazo 3 and ammonium molybdate complex colorimetric techniques, Roche Diagnostics respectively). Albumin was measured by immunoturbidimetry technique (Roche Diagnostics). Arterial blood gas analysis was performed using the GEM® Premier™ 3000 (Instrumentation Laboratory Co, Paris, France) with a preheparinized 3 mL blood gas syringe (RAPI-DLyte®, Siemens Healthcare Diagnostic Inc, USA). Ionized calcium and lactate concentrations were determined with the GEM® Premier 3000. To ensure accurate measurement, the blood gas analyzer was calibrated several times a day.

Acid-base calculations

Bicarbonate and SBE were calculated using the Henderson-Hasselbach and Van Slyke equations, respectively [3,19]. The AG was calculated as follows [20]:

$$AG = [Na^+] + [K^+] - [HCO_3^-] - [Cl^-].$$

AG was corrected for the effect of abnormal albumin concentration [12] and lactate using the formula:

$$AGcorr = AG + 0.25 \times (45 - [\text{albumin in g/L}]) - [\text{lactate mEq/L}].$$

Physicochemical analysis was performed using the Stewart equations [8] modified by Figge et al. [11,13] to consider the effects of plasma proteins. The apparent SID (SID_{app}) is the difference between the sum of all measured strong cations and strong anions as follows (all concentrations in mEq/L):

$$SID_{ai} = [Na^+] + [K^+] + [Ca^{2+}] + [Mg^{2+}] - [Cl^-]$$

The strong ion gap (SIG) was calculated as follow:

$$SIG = SID_{app} - [\text{lactate}^-] -$$
$$\{[HCO_3^-] + (\text{albumin} \times 0.123 \times [pH - 0.631]) + \text{phosphate} \times 0.309 \times [pH - 0.469]\}$$

where HCO_3^- and lactate are in mEq/L, albumin in g/L, and phosphate in mmol/L.

Healthy volunteers and references data

Reference values for the SIG and SID_{app} were previously obtained from arterial blood samples of 13 healthy volunteers [16]. Data in the present study were similarly treated and analyzed. The normal values considered were those between the 2.5 and 97.5 percentiles of values from the healthy volunteers. Thus, the range of normal values for chloride was [99–104] mEq/L [16]. We defined SID_{app} acidosis and SID_{app} alkalosis, that are, metabolic acidosis and alkalosis attributable to disturbances in the inorganic ions, as a SID_{app} value below the 2.5th percentile (42.7 mEq/L) and as a SID_{app} value above 97.5th percentile (47.5 mEq/L) respectively. Also, a SIG value above 8 mEq/L was considered elevated, indicating the presence of unmeasured anions [16].

Patient subgroup division and sequence of analysis

Patients admitted during the first 5 months randomly constituted the modeling group (n = 161), and patients admitted during the last 6 months constituted the cross-validation group (n = 180). Data of the modeling group were used as the basis on which to build a mathematical linear model to derive an equation describing the relationship between SID_{app} and ($[Na^+] - [Cl^-]$) difference, and between SIG and AG_{corr}.

The linear regression models were then tested in the cross-validation group. The regression coefficients produced by the analysis in the modeling group were applied to all initial measurements in a cross-validation group to calculate the predicted values of SID_{app} and SIG. The predicted values and actual values were then compared through correlation and agreement analysis. A large discrepancy between R^2 (Pearson correlation coefficient) for the cross-validation and modeling groups indicated overfitting and lack of generalizability of the results of the analysis [21].

Statistical analysis

The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Proportions were used as descriptive statistics for categorical variables. Analysis of the discrete data was performed by Chi-square test. Continuous data that were not distributed normally were compared using the Mann-Whitney U test; otherwise the Student t test was applied. The mathematical model was built using simple linear regression analysis. Outliers were the cause of concern if more than 5% of cases have standardized residuals with an absolute value greater than 2 [22]. Influence cases were considered if Cook's distance values exceeding 1 [22]. The assumption of homoscedasticity was tested by plotted the standardized residuals values against the standardized predicted values of the dependent variable. To test the

assumption of the normality of residuals, we looked at the histogram of the standardized residual. The assumption of independent residuals was tested with the Durbin-Watson test [22]. The correlation analysis was carried out with Pearson test. Agreement and limits of agreement of 95% were analyzed with the Bland-Altman plot [23] and intraclass correlation coefficient (ICC) [24] for continuous data, and with Kappa coefficient for categorical data [25]. Data were analyzed for the overall cross-validation group and three subgroups: a metabolic acidosis group (SBE<−2 mEq/L), a reference range group (−2 mEq/L≤SBE≤+2 mEq/L) and a metabolic alkalosis group (SBE>+2 mEq/L) [26]. Agreements were also analyzed in the septic shock group with or without acute kidney injury (serum creatinine>1.5 mg/dl) and with or without acute respiratory failure (pH<7.35 and $PaCO_2$>45 mmHg). We used these groups to examine the possibility that different acid-base states may affect the agreement. Sensitivity and specificity, positive and negative likelihood ratios (LHR^+ and LHR^-, respectively), as well as accuracy (the area under the receiver operator characteristic (ROC) curve with a 95% confidence interval [CI]), were calculated to predict the accuracy of diagnosing SID_{app} acidosis, SID_{app} alkalosis, and increased SIG with each surrogate. We calculated the cutoff points with the greatest accuracy using Youden's index [27]. Statistical analyses were performed using SPSS (SPSS for windows release 17.0, Chicago, IL). A value of p<0.05 was considered statistically significant. All reported P values are 2-sided.

Results

The general characteristics of patients, including reasons for ICU admission, clinical outcomes, ICU support measures, and laboratory data for the modeling and cross-validation groups are shown in Table 1.

[Na$^+$] – [Cl$^-$] difference as a surrogate of SID$_{app}$ in the modeling group

The R^2 of the regression analysis in the modeling group with SID_{app} as a dependent variable and [Na$^+$] – [Cl$^-$] difference as independent variable was 0.973 with the F statistics of 5722.33 (P<0.0001). There were 9 cases out of 161 (5.5%) with standardized residuals outside the limits of ±2. Therefore, our sample appears to conform to what we would expect for a fairly accurate model. Moreover, none of these 9 cases had a Cook's distance greater than 1 and so none of the cases had an undue influence on the model. The points of the graph of standardized residuals against standardized predictive values were randomly and evenly dispersed throughout the plot, demonstrating that the assumption of homoscedasticity had been met. Moreover, the histogram of the standardized residuals looked like a bell-shaped curve indicating that the assumption of linearity had also been met. The Durbin-Watson test of the model was 1.9 indicative of the independence of residuals. The equation of the model can be written as follows:

$$SID_{app}(mEq/L) = 8.647(95\%CI : 7.787 - 9.507) +$$
$$[0.976(95\%CI : 0.95 - 1) \times ([Na^+] - [Cl^-])], mEq/L$$

The effect of SID_{app} on SBE ($R^2 = 0.57$) was not different from that of [Na$^+$] – [Cl$^-$] on SBE ($R^2 = 0.56$).

The accuracy of [Na$^+$] – [Cl$^-$] to diagnose SID_{app} acidosis and alkalosis was excellent (0.992; 95%CI: 0.963–1 and 0.998; 95%CI: 0.972–1, respectively). Youden's index was used to determine that

the best values of [Na$^+$] – [Cl$^-$] to predict SID_{app} acidosis (<42.7 mEq/L) and alkalosis (>47.5 mEq/L) were 34 mEq/L and 38 mEq/L, respectively. A value of [Na$^+$] – [Cl$^-$]≤34 mEq/L predicted SID_{app} acidosis (n = 110) with sensitivity of 94.5% (95% CI: 88.5–98), specificity of 98% (95% CI: 89–100), LHR^+ of 52 (95% CI: 13.16–205.4) and LHR^- of 0.06 (95% CI: 0.026–0.12). A value of [Na$^+$] – [Cl$^-$]>38 mEq/L predicted SID_{app} alkalosis (n = 15) with sensitivity of 100% (95% CI: 77–100), specificity of 96 (95%CI: 86–96), LHR^+ of 24.3 (95% CI: 2–277), and LHR^- of 0. Table 2 shows the accuracy, sensitivity, specificity, LHR^+, and LHR^- of SID_{app} surrogate in a subgroup of patients with hyponatremia ([Na$^+$]<135 mEq/L).

AG$_{corr}$ as a surrogate of SIG in the modeling group

The R^2 of the regression analysis in the modeling group with SIG as a dependent variable and AG_{corr} as independent variable was 0.96 with the F statistics of 3606 (P<0.0001). There were 10 cases out of 161 (6%) with standardized residuals outside the limits of ±2. Moreover, none of these 10 cases had a Cook's distance grater than 1. The Durbin-Watson test of the model was 2, and the pattern of the points in the standardized residuals against standardized predictive values plots was indicative that the assumptions of linearity and homoscedasticity had been met. The equation of the model can be written as follows:

$$SIG(mEq/L) = -8.651(95\%CI : -9.171 - -8.132)$$
$$+0.931(95\%CI : 0.901 - 0.962) \times AG_{corr}(mEq/L)$$

The effect of SIG on SBE ($R^2 = 0.61$) was also not different from that of AG_{corr} on SBE ($R^2 = 0.58$).

The accuracy of AG_{corr} to diagnose SIG acidosis was excellent (0.974; 95%CI: 0.936–0.993). The best value found using Youden's index for AG_{corr} to predict SIG acidosis (>8 mEq/L) was 17 mEq/L. A value of AG_{corr}>17 mEq/L predicted SIG acidosis with sensitivity of 95% (95% CI: 86–99), specificity of 93% (95% CI: 86–97), LHR^+ and LHR^- of 13.5 (95% CI: 6.5–27.2) and 0.05 (95% CI: 0.018–0.16); respectively.

Validation of the SID$_{app}$ and SIG surrogates in the cross-validation group

Applying the linear regression models to the cross-validation group, a predictable measure of SID_{app}, and a predictable measure of SIG were calculated. The similarities between predicted and actual values of SID_{app} and between predicted and actual values of SIG were demonstrated by the excellent correlations and agreements between them (Figure 1 and 2). Furthermore, the ICC was 0.988 (95% CI: 0.984–0.991; P<0.0001) between predicted and observed values of SID_{app}, and 0.980 (95% CI: 0.973–0.985; P<0.0001) between predicted and observed values of SIG. These findings were similar in the three subgroups according to SBE (Table 3) and in septic shock patients with and without acute kidney injury and with and without acute respiratory failure (Table 4). By using all first and second data in the cross-validation group, we found an excellent correlation between changes in measured SIG and predicted SIG ($R^2 = 0.94$; P<0.0001), and between changes in measured SID_{app} and predicted SID_{app} ($R^2 = 0.93$; P<0.0001).

The best cutoff values of [Na$^+$] – [Cl$^-$] to diagnose SID_{app} acidosis and alkalosis found in the model group were applied to the cross-validation group for validation. In the first measurements of the cross-validation group, kappa coefficient between [Na$^+$] – [Cl$^-$] values [low (≤34 mEq/L), normal (35–38 mEq/L), high (>38 mEq/L)] and SID_{app} values [low (<42.7 mEq/L), normal

**Table 1.** Patients' admission characteristics, support, outcomes, and laboratory data.

	Modeling group (n = 161)	Validation group (n = 180)	p
Age, y	64 [53–74]	62 [48–72]	0.3
SAPS II (mean ± SD)	56.5±19	54±17	1
Male sex, n (%)	89 (55)	106 (59)	0.5
Mechanical ventilation, n (%)	143 (89)	162 (90)	1
Renal replacement, n (%)	13 (8)	16 (9)	0.99
Vasopressors, n (%)	66 (41)	72 (40)	0.97
ICU survivors, n (%)	108 (67)	126 (70)	0.7
Length of ICU stays, day	11±10	10±9	0.96
Reason of admission, n (%)			
Respiratory failure	50 (31)	54 (30)	0.75
Septic shock	71 (44)	81 (45)	0.8
Postoperative	34 (21)	36 (20)	0.92
Others	6 (4)	9 (5)	0.99
Admission laboratory data			
Na^+, mEq/L	138 [135–142] (124, 152)	138 [135–140] (106, 160)	0.43
K^+, mEq/L	3.8 [3.4–4] (2.3, 5.3)	3.8 [3.6–4.3] (2.7, 7.5)	0.05
Cl^-, mEq/L	105 [101–109] (84, 120)	103 [100–107] (69, 117)	0.014
Ca^{2+}, mEq/L	2.26 [2.14–2.4] (1.34, 3.3)	2.22 [2.1–2.32] (1.34, 3.3)	0.019
Mg^{2+}, mEq/L	1.72 [1.48–1.97] (0.9, 3.12)	1.64 [1.4–1.9] (0.9, 3.28)	0.019
PO_4, mg/L	28 [21–38] (6, 91)	32 [24–43] (3, 104)	0.03
Albumin, g/L	24±5 (10, 37)	25±6 (10, 39)	0.12
Creatinine, mg/dL	1.15 [0.7–1.9] (0.3, 7.2)	1.2 [0.8–2.3] (0.25, 7.3)	0.3
Creatinine>2.5 mg/dL, n (%)	29 (18)	39 (22)	0.5
Lactate, mEq/L	1.2 [0.8–1.7] (0.3, 15)	1.3 [0.9–2.2] (0.2, 15)	0.13
pH	7.41 [7.35–7.45] (7.03, 7.65)	7.39 [7.33–7.46] (6.92, 7.68)	0.037
SID_{app}, mEq/L	40.6 [38–43.7] (29, 60)	42 [39–44.4] (27, 61)	0.02
SIG, mEq/L	6.5 [4–9] (−6, 21)	6.8 [4–10.6] (−8, 22)	0.11
$PaCO_2$, mmHg	37 [32–45] (19, 100)	38 [33–44] (19, 123)	0.38
AG_{corr}, mEq/L	16.2 [13.2–18.7] (4, 30)	16.8 [14–20.5] (2.5, 32)	0.03
$[Na^+] - [Cl^-]$, mEq/L	33 [30–36] (20, 53)	34 [31–37] (21, 54)	0.025
SBE, mEq/L	−0.55 [−3.6–4.3] (−23, 29)	−0.83 [−5–4] (−23, 29)	0.23

SAPS, simplified Acute Physiology Score; SID_{app}, apparent strong ion difference; SIG, strong ion gap; AG_{corr}, anion gap corrected for albumin and lactate; SBE, standard base excess. Values are expressed as medians [interquartile range, 25–75] and (minimum, maximum) unless otherwise stated.
doi:10.1371/journal.pone.0056635.t001

Table 2. Sensitivity, specificity, likelihood ratios, and accuracy of apparent strong ion difference (SID_{app}) surrogate in the presence of Hyponatremia (Na^+<135 mEq/L) (n = 57).

	SID_{app} acidosis n = 43 (75.4)	SID_{app} alkalosis n = 2 (3.5)
$[Na^+] - [Cl^-]$, cutoff	≤34 mEq/L	>38 mEq/L
Sensitivity (%), (95% CI)	95 (84–99)	100 (19–100)
Specificity (%), (95% CI)	93 (66–99)	98 (90–100)
LHR+, (95% CI)	13.35	55
LHR−, (95% CI)	0.05	0
Accuracy, (95% CI)	0.986 (0.911–0.997)	1 (0.937–1)

LHR+, positive likelihood ratio; LHR−, negative likelihood ratio; CI, confidence interval.
doi:10.1371/journal.pone.0056635.t002

A

A

B

B
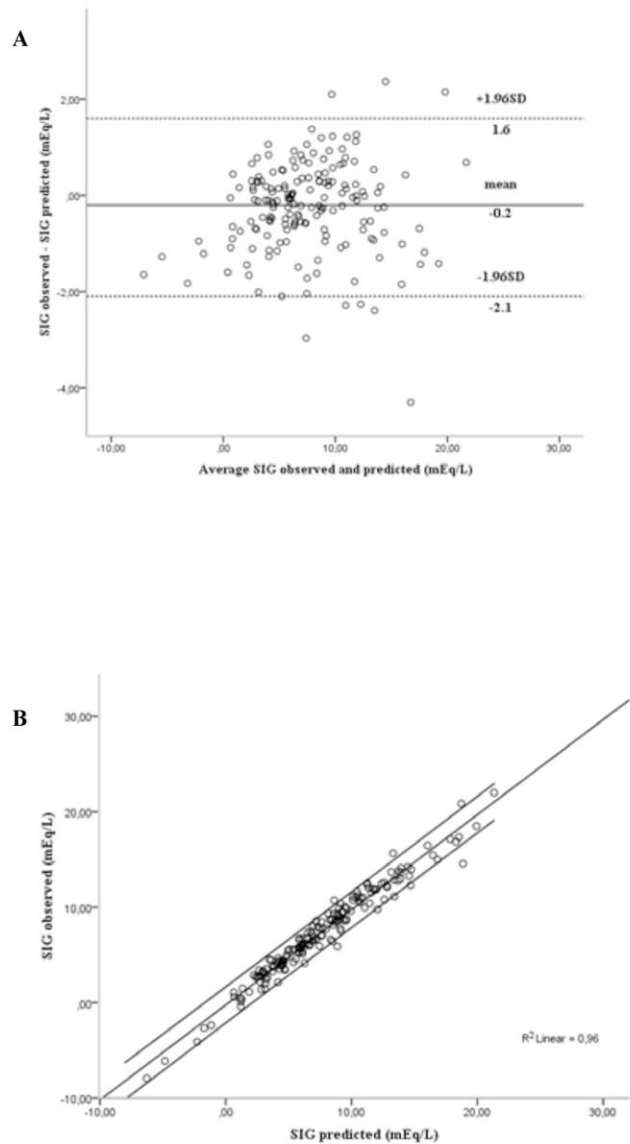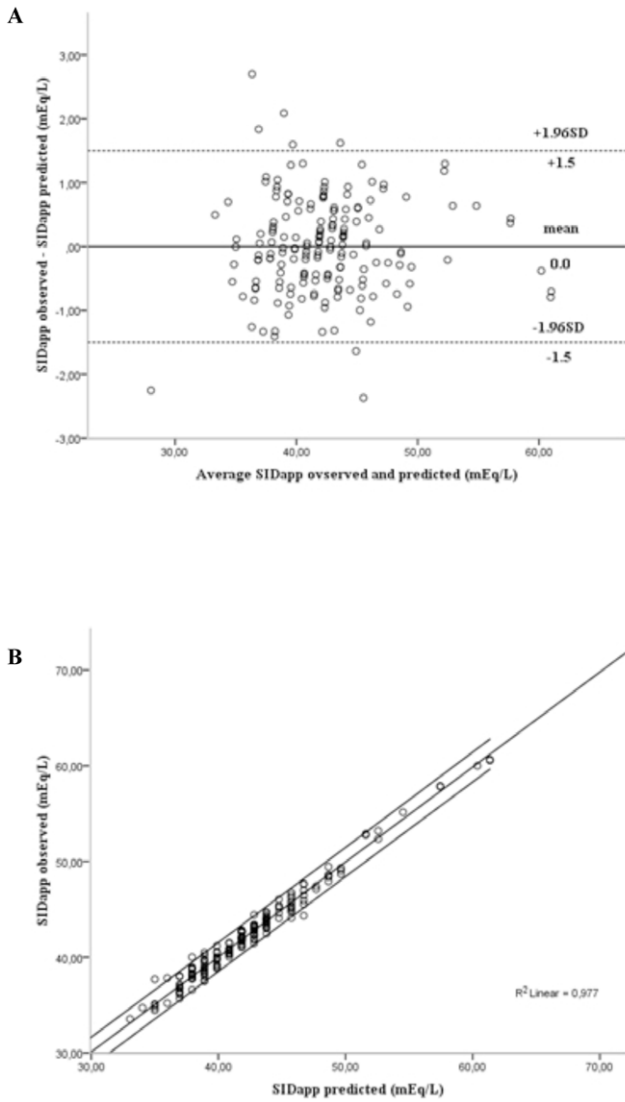
Figure 1. Correlation and agreement between observed and predicted apparent strong ion difference (SIDapp) in the cross-validation group. Panel A shows the agreement between observed and predicted SIDapp (bias = 0, limits of agreement 95% = −1.5 to 1.5 mEq/L). Panel B shows the correlation between observed and predicted SIDapp (R2 = 0.977, P<0.0001).
doi:10.1371/journal.pone.0056635.g001

Figure 2. Correlation and agreement between observed and predicted strong ion gap (SIG) in the cross-validation group. Panel A shows the agreement between observed and predicted SIG (bias = −0.2, limits of agreement 95% = −2.1 to 1.6 mEq/L). Panel B shows the correlation between observed and predicted SIG (R2 = 0.96, P<0.0001).
doi:10.1371/journal.pone.0056635.g002

(42.7–47.5 mEq/L), high (>47.5 mEq/L)] was very good (0.842, 95%CI: 0.786–0.916; $P<0.0001$). The same analysis was done between AG_{corr} (cutoff value >17 mEq/L) and SIG values (>8 mEq/L) in the first measurements of the cross-validation group. Kappa coefficient between these variables was also very good (0.85, 95%CI: 0.773–0.928; $P<0.0001$).

Negative values of SIG were found in 10 (3%) of the 341 patients. All these patients had severe metabolic alkalosis (SBE = 22.3±6 mEq/L and $[HCO_3^-]$ = 48±7 mEq/L), associated with hypochloremia ($[Cl^-]$ = 93±7 mEq/L).

Discussion

The main findings of this study were that: 1) SID_{app} and SIG were well predicted by $[Na^+]$ – $[Cl^-]$ difference and AG_{corr} values respectively. These were confirmed by the excellent correlation

and good agreement found between measured and predicted values of Stewart's variables in the cross-validation group, and in the three subgroups classified according to SBE. 2) The accuracies of $[Na^+]$ – $[Cl^-]$ and AG_{corr} in revealing metabolic disturbance according to SID_{app} and to SIG (respectively) were very high. Furthermore, these findings were confirmed by finding that the kappa coefficients between $[Na^+]$ – $[Cl^-]$ and SID_{app} values, and between AG_{corr} and SIG values were very good in the cross-validation group.

Serum bicarbonate, SBE, and AG are commonly used to assess acid-base disorders [9]. However, it is recognized that this method can fail to identify the complex metabolic disturbances seen in critically ill patients, and so is generally inadequate in explaining them [14]. An alternative approach is the application of basic physicochemical principles of aqueous solutions to blood. Stewart

Table 3. Subgroups analysis of acid-base variables, agreements and intraclass correlation coefficients between observed and predicted values of SID$_{app}$ and of SIG, and kappa coefficients between SID$_{app}$ and its surrogate and between SIG and its surrogate in the cross-validation group.

	Metabolic acidosis (n = 75)	Reference range (n = 44)	Metabolic alkalosis (n = 61)
pH	7.32 (6.92–7.47)	7.4 (7.29–757)	7.45 (7.28–7.68)
HCO$_3$, mEq/L	19 (2–25)	24 (21–28)	29 (25–56)
PaCO$_2$, mmHg	34 (19–123)	39 (25–57)	42 (28–118)
[Na$^+$] – [Cl$^-$], mEq/L	31 (21–41)	34 (27–42)	36 (29–54)
SID$_{app}$, mEq/L	39 (27–48)	42 (35–49)	44 (37–60)
SIG, mEq/L	11 (3–22)	6.5 (3–16.5)	4 (−8–14.6)
AG$_{corr}$, mEq/L	21 (12–32)	16.4 (12–26.5)	14 (3.5–29.5)
Albumin, g/L	23 (10–39)	24 (17–38)	26 (14–39)
ICC between observed and predicted SID$_{app}$, 95%CI	0.968 (0.950, 0.980)	0.984 (0.970, 0.991)	0.992 (0.986, 0.995)
Agreement between observed and predicted SID$_{app}$	0.1 (−1.7, 1.9)	−0.12 (−1.18, 0.95)	−0.02 (−1.37, 1.33)
ICC between observed and predicted SIG, 95%CI	0.969 (0.951, 0.981)	0.955 (0.919, 0.976)	0.966 (0.943, 0.979)
Agreement between observed and predicted SIG	−0.1 (−2.1, 1.8)	−0.2 (−1.83, 1.43)	−0.4 (−2.3, 1.5)
Kappa between SID$_{app}$ and [Na$^+$] – [Cl$^-$], 95%CI	0.848 (0.702, 0.993)	0.755 (0.553, 0.957)	0.819 (0.692, 0.945)
Kappa between SIG and AG$_{corr}$, 95%CI	0.735 (0.533, 0.938)	0.807 (0.627, 0.987)	0.743 (0.469, 1)

SID$_{app}$, apparent strong ion difference; SIG, strong ion gap; AG$_{corr}$, anion gap corrected for albumin and lactate; ICC, intraclass correlation coefficient; CI, confidence interval. Metabolic acidosis = SBE<−2 mEq/L, reference range = −2 mEq/L≤SBE≤+2 mEq/L, and metabolic alkalosis = SBE>+2 mEq/L. Agreement is expressed as bias, (95% limits of agreement). All others data are expressed as median with range (minimum, maximum).
doi:10.1371/journal.pone.0056635.t003

method allows for the quantification of pH variations in proportion of changes in the independent variables [8]. However, Stewart developed his mathematical model in a flask, and there are certain points to note when applying this model to human plasma. First, the PaCO$_2$ is an independent variable in an "open" system, where the total carbon dioxide is not fixed because it is in equilibrium with alveolar gas. However, this does not strictly apply to venous blood and fluid within the tissues, where the system is closed and the total carbon dioxide content rather than PaCO$_2$ is the independent variable [9]. Second, no quantitative assessment of the secondary responses to primary changes in acid-base status is offered by the physicochemical approach [28]. Nevertheless, several studies [15,16,17,29] have demonstrated that the Stewart's approach to acid-base disturbances allows the differentiation between tissue acidosis and hyperchloremic acidosis, and then results in identification of more patients with major acid-base disorders than the traditional evaluation.

Quantitatively, a change in the strong ion composition leading to lower SID will increase [H$^+$] and causing SID$_{app}$ acidosis while an increase in SID will decrease [H$^+$] and causing SID$_{app}$ alkalosis. Hyperchloremic acidosis therefore causes acidosis by decreasing SID$_{app}$ and not through hyperchloremia alone. Indeed, normochloremia can occur alongside hyponatremia and result in acidosis by decreasing SID$_{app}$, and hypernatremia can occur alongside hyperchloremia without acidosis (no change in SID$_{app}$) [30]. At the other end of the spectrum, alkalosis may thus occur with both hypochloremia and hyperchloremia, with the latter occurring in the presence of greater hypernatremia (greater SID$_{app}$) [31]. These highlight the importance of SID$_{app}$ in our understanding and management of complex acid-base disorders in critically ill patients.

Nevertheless, ionized calcium and magnesium concentrations (2 components of SID$_{app}$) are not included in routine chemistry profiles in ICU. Moreover, calculation of SID$_{app}$ is time-consuming and is therefore not convenient for use in daily

Table 4. Subgroups analysis in the septic shock patients of the cross-validation group according to the presence of acute kidney injury and of acute respiratory failure.

	AKI (n = 32)	Non-AKI (n = 49)	ARF (n = 20)	Non-ARF (n = 61)
ICC between observed and predicted SID$_{app}$, 95%CI	0.990 (0.983, 0.993)	0.989 (0.983, 0.992)	0.996 (0.993, 0.998)	0.982 (0.974, 0.987)
Agreement between observed and predicted SID$_{app}$	0.21 (−1.20, 1.62)	−0.13 (−1.62, 1.35)	0.27 (−0.80, 1.35)	−0.06 (−1.61, 1.61)
ICC between observed and predicted SIG, 95%CI	0.981 (0.967, 0.989)	0.981 (0.970, 0.988)	0.977 (0.956, 0.988)	0.976 (0.967, 0.983)
Agreement between observed and predicted SIG	−0.30 (−1.44, 0.84)	−0.21 (−1.73, 1.32)	−0.62 (−2.34, 1.10)	−0.15 (−1.99, 1,7)
Kappa between SID$_{app}$ and [Na$^+$] – [Cl$^-$], 95%CI	0.879 (0.704, 1)	0.817 (0.670, 0.964)	0.918 (0.689, 1)	0.812 (0.679, 0.944)
Kappa between SIG and AG$_{corr}$, 95%CI	0.732 (0.521, 0.931)	0.842 (0.775, 1)	0.817 (0.584, 1)	0.944 (0.752, 1)

AKI, acute kidney injury; ARF, acute respiratory failure, SID$_{app}$, apparent strong ion difference; SIG, strong ion gap; AG$_{corr}$, anion gap corrected for albumin and lactate; ICC, intraclass correlation coefficient; CI, confidence interval. Agreement is expressed as bias, (95% limits of agreement).
doi:10.1371/journal.pone.0056635.t004

practice. Thus, a simplified equation is suitable for use at the bedside. Chloride and sodium are the most abundant extracellular ions and then the major contributors to the SID_{app}. Previous studies have shown that in plasma, the SID_{app} is largely the difference between sodium cations and chloride anions [32,33]. Recently, the difference between $[Na^+]$ and $[Cl^-]$ was found to have a good correlation and short limit of agreement with SID_{app} [18]. However, in these studies there was no independent sample of patients to validate these findings. In our study, we built a linear regression equation to assess the relationship between SID_{app} and $[Na^+] - [Cl^-]$ in the modeling group of patients. We found that SID_{app} can well be predicted from $[Na^+] - [Cl^-]$. Furthermore, the effect of SID_{app} on SBE was not different from that of $[Na^+] - [Cl^-]$ on SBE.

In the same way, we found that SIG can well be predicted from AG_{corr}, and the effect of SIG on SBE was also not different from that of AG_{corr} on SBE. Our findings are in line with previous researches [16,17,26], which found a high correlation and good agreement between AG_{corr} and SIG.

To test the mathematical models resulting from the modeling group analysis, the equations that have been built were used to predict the values of SID_{app} and SIG in the cross-validation group. We found an excellent correlation and good agreement and precision between predicted and measured SID_{app} and between predicted and measured SIG (Figure 1 and 2). These were still true in the three subgroups classified according to SBE (Table 3). In addition, the temporal evolution of the predicted SID_{app} and SIG was well correlated with that of the observed SID_{app} and SIG. Therefore, by these findings we have demonstrated that these models are accurate and can be generalized to other samples.

Moreover, the accuracies of $[Na^+] - [Cl^-]$ and AG_{corr} in revealing SID_{app} metabolic disorders and SIG acidosis (respectively) were high in the modeling group. Furthermore, we validated the cutoff values by finding a very good kappa coefficient between $[Na^+] - [Cl^-]$ and SID_{app} values, and between AG_{corr} and SIG values in the cross-validation group. Our results are in accordance with those of Nagaoka et al. [18]. Nevertheless, the discrepancy between the cutoff values of $[Na^+] - [Cl^-]$ to predict SID_{app} acidosis in that study (32.5 mEq/L) and in ours could be explained by the poor reproducibility of electrolyte measurements between different laboratory analyzers [34].

Patients with hyponatremia can have SID_{app} acidosis with normal serum chloride levels or can have hypochloremia without SID_{app} alkalosis [30]. The cutoff values of $[Na^+] - [Cl^-]$ retrieved from the ROC curve were able to identify SID_{app} acidosis and alkalosis with high specificity and sensitivity in patients with hyponatremia (Table 2). We found only 10 patients with hypernatremia ($Na^+ > 145$ mEq/L) that is why we could not do the same analysis with these patients.

The observed negative values of SIG in our patients may mean an error in measurement or the presence of unmeasured cations, which is rare even in critically ill patients. We think that laboratory error was unlikely since: (1) sodium and chloride were both measured using ion selective electrodes, and (2) no unexpected results were found in other patients. Moreover, all these patients suffered from severe metabolic alkalosis associated with hypochloremia. Therefore, the only feasible explanation for this rare finding was the accumulation of unmeasured cations. Medical literature involving unmeasured cations is poor [15,35] and has been described in patients with chronic renal failure (accumulation of guanidines) [36], lithium intoxications [37], and paraproteinemias (positively charged gammaglobulins) [38]. In our patients, there was no history of medication abuse, and we did not investigate the presence of gammopathy.

Our study has several limitations. First, it has been done in a large sample of critically ill patients from a single unit. Our findings might not apply to other populations. However, our ICU admits a variety of medical and surgical patients, and our population is likely to be representative of other general ICU populations. Second, our results might not be applicable in patients with hypernatremia due to their small number.

Conclusion

The present study demonstrates that SID_{app} and SIG can be substituted by the difference between $[Na^+]$ and $[Cl^-]$ and by the AG_{corr} respectively in the diagnosis and management of acid-base disorders in critically ill patients. In this manner, the use of these surrogates in metabolic acid-base disorders is fast and simple and may prevent the need of the complex calculations of Stewart's method.

Acknowledgments

Author Contributions

Conceived and designed the experiments: JM SB. Performed the experiments: JM SB ML FP GG LT DT. Analyzed the data: JM. Contributed reagents/materials/analysis tools: JM SB. Wrote the paper: JM.

References

1. Gunnerson KJ, Kellum JA (2003) Aid-Base and electrolyte analysis in critically ill patients: are we ready for the new millennium? Curr Opin Crit Care 9: 468–473.
2. Gauthier PM, Szerlip HM (2002) Metabolic acidosis in the intensive care unit. Crit Care Clin 18: 289–308.
3. Henderson LJ (1908) The theory of neutrality regulation in the animal organism. Am J Physiol 21: 427–428.
4. Van Slyke DD, Wu H, McLean FC (1923) Studies of gas and electrolyte equilibria in the blood. V. Factors controlling the electrolyte and water distribution in the blood. J Biol Chem 56: 765–849.
5. Astrup P (1956) A simple electrometric technique for the determination of carbon dioxide tension in blood and plasma, total content of carbon dioxide in plasma, and bicarbonate content in 'separated' plasma at a fixed carbon dioxide tension (40 mmHg). Scand J Clin Lab Invest 8: 33–43.
6. Andersen OS, Engel K, Jorgensen K, Astrup P (1960) A micro method for determination of pH, carbon dioxide tension, base excess and standard bicarbonate in capillary blood. Scand J Clin Invest 12: 172–176.
7. Fencl V, Leith DE (1993) Stewart's quantitative acid-base chemistry: applications in biology and medicine. Respir Physiol 91: 1–16.
8. Stewart PA (1983) Modern quantitative acid-base chemistry. Can J Physiol Pharmacol 61:1444–1461.
9. Sirker AA, Rhodes A, Grounds RM, Bennett ED (2002) Acid-base physiology: the 'traditional' and the 'modern' approaches. Anaesthesia 57: 348–356.
10. McAuliffe JJ, Lind LF, Leith DE, Fencl V (1986) Hypoproteinemic alkalosis. Am J Med 81: 86–90.
11. Figge J, Rossing TH, Fencl V (1991) The role of serum proteins in acid-base equilibria. J Lab Clin Med 117: 453–467.
12. Figge J, Jabor A, Kadza A, Fencl V (1998) Anion gap and hypoalbuminemia. Crit Care Med 26: 1807–1810.
13. Figge J, Mydosh T, Fencl V (1992) Serum proteins and acid-base equilibria: a follow-up. J Lab Clin Med 120: 713–719.
14. Fencl V, Jabor A, Kazda A, Figge J (2000) Diagnosis of metabolic acid-base disturbances in critically ill patients. Am J Resp Crit Care Med 162: 2246–2251.
15. Boniatti MM, Cardoso PR, Castilho RK, Vieira SR (2009) Acid-base disorders evaluation in critically ill patients: we can improve our diagnostic ability. Intensive Care Med 35: 1377–1382.

16. Mallat J, Michel D, Salaun P, Thevenin D, Tronchon L (2012) Defining metabolic acidosis in patients with septic shock using Stewart approach. Am J Emerg Med 30: 391–398.

17. Moviat M, Van Haren F, van der Hoeven H (2003) Conventional or physicochemical approach in intensive care unit patients with metabolic acidosis. Crit Care 7: R41–R45.

18. Nagaoka D, Nassar Junior AP, Maciel AT, Taniguchi LU, Noritomi DT, et al. (2010) The use of sodium-chloride difference and chloride-sodium ratio as strong ion difference surrogates in the evaluation of metabolic acidosis in critically ill patients. J Crit Care 25: 525–531.

19. Siggaard-Anderson O (1977) The Van Slyke equation. Scand J Clin Lab Invest 37: 15–20.

20. Oh MS, Carroll HJ (1977) The anion gap. N Engl J Med 297: 814–817.

21. Tabachnick BG, Fidell LS (2007) Multiple regression. In: Using Multivariate Statistics 5th edition. pp. 117–194.

22. Field A (2009) Regression. In: Discovering statistics using SPSS 3th edition. pp.197–263.

23. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1: 307–310.

24. Shrout PE, Fleiss JL (1979) Intraclass correlations: uses in assessing rater reliability. Psychol Bull 86: 420–428.

25. Cohen J (1960) A coefficient of agreement for nominal scales. Educ Psychol Meas 20: 37–46.

26. Park M, Taniguchi LU, Noritomi DT, Libório AB, Maciel AT, et al. (2008) Clinical utility of standard base excess in the diagnosis and interpretation of metabolic acidosis in critically ill patients. Braz J Med Biol Res 41: 241–249.

27. Bewick V, Cheek L, Ball J (2004) Statistics review 13: receiver operating characteristic curves. Crit Care 8: 508–512.

28. Adrogué HJ, Madias NE (2010) Secondary responses to altered acid-base status: the rules of engagement. J Am Soc Nephrol 21: 920–923.

29. Murray DM, Olhsson V, Fraser JI (2004) Defining acidosis in postoperative cardiac patients using Stewart's method of strong ion difference. Pediatr Crit Care Med 5: 240–245.

30. Story DA (2004) Hyperchloraemic acidosis: another misnomer? Crit Care Resusc 6: 188–192.

31. Yunos NM, Bellomo R, Story D, Kellum J (2010) Bench-to-bedside review: Chloride in critical illness. Crit Care 14: 226. doi:10.1186/cc9052

32. Story DA, Morimatsu H, Bellomo R (2004) Strong ions, weak acids and base excess: a simplified Fencl-Stewart approach to clinical acid-base disorders. Br J Anaesth 92: 54–60.

33. Gilfix BM, Bique M, Magder S (1993) A physical chemical approach to the analysis of acid-base balance in the clinical setting. J Crit Care 8: 187–197.

34. Nguyen BV, Vincent JL, Hamm JB, Abalain JH, Carre JL, et al. (2009) The reproducibility of Stewart parameters for acid-base diagnosis using two central laboratory analyzers. Anesth Analg 109: 1517–1523.

35. Maciel AT (2009) Severe metabolic alkalosis due to the combination of unmeasured cations and hypochloraemia in a patient with gastroparesia and frequent emesis. BMJ Case Rep doi:10.1136/bcr.09.2008.1011

36. Parikh C, Gyamlani G, Panlilio N, Carvounis CP (2001) Unmeasured cations: probable cause of relatively low anion gap in chronic renal failure. Ren Fail 23:91–96.

37. Kelleher SP, Raciti A, Arbeit LA (1986) Reduced or absent serum anion gap as a marker of severe lithium carbonate intoxication. Arch Intern Med 146: 1839–1840.

38. Mansoor S, Siddiqui I, Adil S, Nabi Kakepoto G, Fatmi Z, et al. (2007) Anion gap among patients of multiple myeloma and normal individuals. Clin Biochem 40:226–229.