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Physicochemical Approach to Determine the Mechanism for Acid–Base Disorders in 793 Hospitalized Foals

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Background: The quantitative effect of strong electrolytes, unmeasured strong anions (UAs), pCO_2, and plasma protein concentrations in determining plasma pH can be demonstrated using the physicochemical approach. Plasma anion gap (AG) and strong ion gap (SIG) are used to assess UAs in different species.

Hypotheses: Strong ions are a major factor influencing changes in plasma pH of hospitalized foals. AG and SIG accurately predict severe hyper-L-lactatemia ([L-lac$^-$] > 7 mmol/L).

Animals: Seven hundred and ninety three hospitalized foals < 7 days old.

Methods: Retrospective study. The relationship between measured pH and physicochemical variables, and the relationship between plasma [L-lac$^-$] and AG and SIG, were determined using regression analyses. Optimal AG and SIG cut points to predict hyper-L-lactatemia were identified using an ROC curve analysis.

Results: Combined, the measured strong ion difference and SIG accounted for 54–69% of the changes in the measured arterial pH of hospitalized foals. AG and SIG were significantly associated with plasma [L-lac$^-$] ($P < .0001$). The receiver operator characteristics (ROC) AUC of AG and SIG for prediction of severe hyper-L-lactatemia were 0.89 (95%CI, 0.8–0.95; $P < .0001$) and 0.90 (95%CI, 0.81–0.96; $P < .0001$), respectively. Severe hyper-L-lactatemia was best predicted by AG > 27 mmol/L (sensitivity 80%, 95%CI, 56–94, specificity 85%, 95%CI, 73–93; $P < .0001$) and SIG <−15 mmol/L (sensitivity 90%, 95%CI, 68–98; specificity 80%; 95%CI, 68–90; $P < .0001$).

Conclusion and clinical relevance: Altered concentrations of strong ions (Na^+, K^+, Cl^-) and UAs were the primary cause of acidemia of hospitalized foals. AG and SIG were good predictors of hyper-L-lactatemia and could be used as surrogate tests.

Key words: Anion gap; Nonvolatile weak acids; Strong ion difference; Strong ion gap; Unmeasured anions.

Hospitalized foals with critical disorders such as sepsis, pneumonia, enterocolitis, neonatal encephalopathy (NE), and prematurity/dysmaturity frequently develop acid–base, fluid, electrolyte and plasma protein disturbances.[1,2] Possible mechanisms for these disturbances include reduced perfusion because of hypovolemia and hypotension,[3–6] activation of hormonal mechanisms including the renin angiotensin aldosterone system and vasopressin,[7–9] tubular damage,[10,11] and perturbation of the hypothalamic–pituitary–adrenal axis.[12] Plasma protein alterations might be caused by increased capillary leakage,[12,13] altered intravascular and tissue albumin distribution, imbalances between albumin synthesis and degradation[14,15] and failure of transfer of passive immunity (FTPI).[2]

The traditional approaches to acid–base determinations are often inadequate to explain the severe disorders present in some critically ill patients. Neither the base excess nor the bicarbonate can explain the cause of a disturbance, only its existence and severity.[16,17] The quantitative physicochemical approach emphasizes the importance of strong ion difference (SID) which is the difference between the charge of plasma strong cations (Na^+, K^+,Ca^{2+}, Mg^{2+} and anions (Cl^-, L-lactate$^-$, sulfate, and ketoacids), p_aCO_2, and total plasma protein concentration (A_{tot}) in determining plasma pH and [HCO_3^-].[18,19] This approach is mechanistic and provides a more in-depth insight into the pathophysiology of acid–base imbalances.[18] The mechanisms responsible for local, regional, and systemic acid–base are incompletely understood and controversy exists in the literature as to what method should be used.[20] Previous studies have demonstrated that p_aCO_2, SID, and A_{tot} are causally related to pH and are mathematically independent determinants of

Abbreviations:

A^-	total net negative charge of plasma proteins
AG	anion gap
A_{tot}	total plasma concentration of nonvolatile weak acids
AUC	area under the curve
FTPI	failure of transfer of passive immunity
H-H	Henderson–Hasselbalch approach
HCO_3^-	bicarbonate
mmol	millimoles
L-lac$^-$	L-lactate
NE	neonatal encephalopathy
p_aCO_2	partial arterial carbon dioxide pressure
ROC	receiver operator characteristics
SID	strong ion difference
SIG	strong ion gap
TPP	total plasma protein
UAs	unmeasured anions

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plasma pH in sick calves with or without diarrhea.[21,22] However, there is a lack of information regarding the quantitative contribution of the physicochemical variables to the changes of plasma pH of equine neonates.

Hypovolemia and hypotension in critically ill foals often results in tissue hypoxia and anaerobic metabolism, reflected by hyper-L-lactatemia. The severity of hyper-L-lactatemia is considered an accurate predictor of mortality in neonatal and adult humans, horses, and cattle.[1,3,23–26] Although recent studies have shown that serial measurement of plasma concentration of L-lactate ([L-lac$^-$]) or measurement of [L-lac$^-$] clearance might enable more accurate prognostication,[3,4] [L-lac$^-$] determination at hospital admission are commonly used as a prognostic indicator. For instance, admission [L-lac$^-$] > 6.9 mmol/L correctly classified 86% of neonatal foals as nonsurvivors.[24]

Clinicians could assume that commonly calculated acid–base parameters, like anion gap (AG) and strong ion gap (SIG) are sufficient alternatives for prediction of hyper-L-lactatemia, but conflicting results regarding the sensitivity and specificity of AG when investigating metabolic acidosis in human, equine, and bovine adult and neonates has been documented.[1,5,25–27] Alternatively, SIG has shown an excellent correlation with [L-lac$^-$] in healthy horses under exercise conditions,[28] and horses with acute diarrhea.[29] But, the sensitivity and specificity of AG and SIG to predict [L-lac$^-$] in hospitalized foals is currently unknown.

The first study objective was to quantitate the effect of physicochemical variables on plasma pH of hospitalized foals. The second objective was to investigate whether AG and SIG could be used as a screening tool for clinically important hyper-L-lactatemia ([L-lac$^-$] > 7 mmol/L) in hospitalized foals. We hypothesized that changes in strong ions (SIDm), p_aCO_2, A_{tot} are the major determinants of plasma pH and that AG and SIG accurately predict clinically important hyper-L-lactatemia in hospitalized foals.

Materials and Methods

Case Selection Criteria and Medical Records Review

Data from medical records of all neonatal foals admitted to the Hofmann Equine Neonatal Intensive Care Unit at the University of Florida from January 1982 through June 2007 were entered into a computerized data-base.[a] Foals were included in the study if: (1) they were younger than 7 days of age, and (2) the measurement of arterial blood gases and basic plasma metabolic profile was performed within the first hour of hospitalization before parenteral fluid therapy administration.

Data Collection

Medical records were systematically reviewed and the following information was recorded: age at admission; breed; sex; year of admission; rectal temperature; respiratory rate (RR, respiration per minute); heart rate (HR, beats per minute); sepsis score;[30] total plasma protein concentrations (mg/dL); plasma sodium (mmol/L), potassium (mmol/L), chloride (mmol/L), L-lactate (mmol/L) (available only for 81 foals admitted between 2005 and 2007), creatinine (mg/dL), total calcium (mg/dL) concentrations; arterial blood pH; p_aCO_2 (mmHg), arterial HCO_3^- (mmol/L); and arterial base excess (mmol/L). Five major diagnostic categories, determined by the admitting clinician, were used for comparison and included: premature/dysmature, NE, sepsis, enterocolitis, and "Other" diagnoses. A diagnosis of sepsis was based on one or both of the following criteria: (1) positive blood culture; (2) sepsis score ≥ 12.[30] All of the foals included in premature/dysmature, NE, enterocolitis, and "Other" diagnoses groups tested negative in the blood culture, had a sepsis score < 12, or both. For instances, if a foal had diarrhea on admission and the blood culture was positive, or had a sepsis score ≥ 12, or both, the foal was included in the sepsis group. Duration of hospitalization was based on either the number of days until the foal was discharged from the hospital or on the number of days until death. Survival was defined as discharge from the hospital.

Calculations

Determination of the SID requires accurate measurement of all strong ions in plasma or serum, including ideally the measurement of unmeasured strong anions. SID has 2 components: measured strong ion difference (SID_m), which was calculated from the measured plasma concentrations of 3 strong ions (Na^+, K^+, Cl^-) as:[18]

$$SID_m = Na + K - Cl$$

and unmeasured strong ion difference (SID_{um}) calculated as:[19]

$$SID_{um} = S \times p_aCO_2 \times 10^{(pH-pK1')} - \frac{A_{tot}}{[(1 + 10^{(pKa-pH)}]} - SID_m$$

using assigned values for S (constant for the solubility of carbon dioxide) of 0.0307 $mmol/L^{-1} \times mmHg^{-1}$ and pK_1' of 6.120.[19]

Strong ion gap was calculated by rearranging the above equation and substituting SID_{um} for SIG as:[19]

$$SIG = \frac{[A_{tot}]}{[(1 + 10^{(pKa-pH)}]} - AG$$

where pK_a (6.65) is the effective dissociation constant of equine plasma weak acids[19] and total plasma concentration of weak acids (A_{tot}) was calculated as:

$$A_{tot}\ (mmol/L) = [0.22 \times (TP)]$$

where total protein is in g/L.[19]
Anion gap was calculated as:[31]

$$AG\ (mmol/L) = ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$$

Statistical Analyses

Data are presented as mean ± SD. Nonnormally distributed data are presented as median and quartiles 25 and 75%. Normality of the data was tested by the Kolmogorov–Smirnov test. Differences between groups were assessed using a paired t-test or the Mann–Whitney U-test depending on the normality of the data. The nonparametric Kruskal–Wallis test was used to examine differences among populations of foals with different clinical diagnoses.[24] The relationship between measured plasma pH and SID_m, SIG, p_aCO_2, and A_{tot} was determined using a forward stepwise regression

($P < .05$ to enter and exit).[21] The assumptions of the forward stepwise regression procedure were evaluated by examining the residual plots. The results of the regression were confirmed using backward elimination.[21]

To investigate whether AG and SIG can be used as a screening tool for clinical important hyper-L-lactatemia ([L-lac$^-$] > 7 mmol/L) in hospitalized foals, a subset of 81 foals, in which L-lactate was determined upon admission, was extracted. Normality of the data was tested by the Kolmogorov–Smirnov test. A simple linear regression was used to evaluate the relationship between plasma [L-lac$^-$] and AG and SIG. Receiver operator characteristics (ROC) curve analysis was used to identify the AG and SIG cut-off that optimized diagnostic sensitivity and specificity for predicting hyperlactatemia ([L-lac$^-$] > 7 mmol/L). Results will be presented as area under the ROC curve as well as the 95% confidence interval (CI). Optimal cut-off values were defined by the points representing the highest concomitant sensitivity and specificity. Differences in the areas under the ROC curve (AUC) of AG and SIG were compared using a nonparametric method using the theory on generalized *U*-statistic.[32] Differences were classified as significant if $P < .05$. Statistical analyses were performed using a commercial statistical software package.[b]

Results

Study Population

Seven hundred and ninety three foals met the inclusion criteria. Breed distribution was similar to that of the general hospital population, with Thoroughbreds (567 foals; 72%) being the most represented. Other breeds included Quarter horse (45; 6%), Arabian (45; 6%), Andalusian, American Miniature Horse, Paint, Paso Fino, Standardbred, and Warmblood. Of the 793 foals, 376 (48%) were female, and 407 (51%) male. Sex was not recorded in ten (1%) foals. The median age at admission was 1 day (0–3). A sepsis score was calculated in 548 foals (70%), with 275 (51%) foals ≥12. Blood culture was performed in 593 foals (75%), with 263 (44%) positive. In total, 417 (53%) of 793 foals had a diagnosis of sepsis. One hundred fifteen (28%) of417 foals were septic based on both sepsis score and blood culture, 160 (38%) based on just sepsis score and, 142 (34%) based on blood culture. Three hundred seventy six (47%) of 793 foals were considered nonseptic. Major diagnostic categories for nonseptic foals included prematurity/dysmaturity (38, 4.7%), NE (82; 10%), enterocolitis (78, 10%), or "other" (178, 22%), including neonatal isoerythrolysis, pneumonia, meconium impaction, FTPI, and uroperitoneum. Five hundred and thirty six foals (68%) survived to hospital discharge; of the nonsurvivors, 170 foals (66%) were euthanized and 87 foals (34%) died.

Biochemical and Acid–Base Analyses Findings

Admission values of creatinine, electrolytes, total plasma proteins, and acid–base variables from foals with different clinical diagnoses are presented in Table 1. Comparison of these variables between septic and nonseptic foals and surviving and nonsurviving foals are presented in Tables S1 and S2, respectively.

Determinants of Acid–Base Disorders in Hospitalized Foals

Regression analysis revealed that alterations in both SID_m and SIG were the most important contributors to changes in plasma pH (r^2: 54–69%; $P < .0001$) (Table 2). SID_m was the most important contributor to the changes of pH in premature foals (r^2: 40%; $P < .0001$) and foals with enterocolitis (r^2: 44%; $P < .0001$), whereas SIG was the most important independent variable associated with changes in plasma pH in foals with NE (r^2: 38%; $P < .0001$). Alteration in p_aCO_2 was an important contributor to changes in the arterial pH (r^2: 15–29%; $P < .0001$), especially in premature foals (r^2: 25% $P < .0001$) and foals with NE (r^2: 25% $P < .0001$). Alterations of A_{tot} concentration were associated with changes in plasma pH, especially in foals with sepsis (r^2: 8.2%; $P < .0001$) and enterocolitis (r^2: 14%; $P < .0001$).

Table 1. Admission venous values of plasma creatinine, electrolytes, and arterial blood gas and acid–base variables of 793 hospitalized foals with different clinical diagnoses.

Variable	Sepsis (n = 417)	Prematurity (n = 38)	NE (n = 82)	Enterocolitis (n = 78)	Others (n = 178)
Creatinine (mg/dL)	2.2 (1.3–4.1)^{a}	2.1 (1.5–4.4)^{ac}	2.6 (1.8–4.6)^{a}	1.5 (1.1–2.6)^{bc}	1.8 (1.2–3.3)^{bc}
Na^+ (mmol/L)	137 (133–142)^{a}	138 (133–141)^{a}	141 (137–145)^{b}	137 (132–142)^{a}	137 (132–142)^{a}
K^+ (mmol/L)	4.0 (3.4–4.5)^{a}	3.9 (3.3–4.7)^{ab}	3.4 (3.0–4.3)^{c}	3.6 (3.3–4.1)^{bc}	3.7 (3.2–4.2)^{b}
Cl^- (mmol/L)	99 (94–104)^{a}	99 (95–102)^{a}	99 (94–104)^{a}	101 (98–108)^{b}	98 (92–102)^{a}
TPP (g/L)	57 (50–65)^{a}	50 (46–57)^{b}	53 (47–57)^{b}	60 (54–66)^{c}	56 (50–60)^{a}
pH	7.33 (7.24–7.40)^{a}	7.36 (7.29–7.38)^{ac}	7.36 (7.27–7.41)^{c}	7.33 (7.23–7.40)^{a}	7.4 (7.36–7.42)^{b}
p_aCO_2 (mmHg)	44 (37–53)^{a}	50 (46–53)^{d}	47 (43–51)^{c}	38 (31–45)^{b}	47 (41–51)^{ac}
HCO_3^- (mmol/L)	24 (18–28)^{a}	26 (23–30)^{bd}	27 (22–29)^{d}	20 (14–26)^{c}	28 (24–31)^{b}
AG (mmol/L)	19 (14–25)^{a}	15 (12–22)^{b}	20 (14–26)^{a}	17 (11–23)^{ab}	15 (11–20)^{b}
SID_m (mmol/L)	43 (37–48)^{a}	42 (39–47)^{a}	46 (42–52)^{b}	38 (33–44)^{c}	42 (38–47)^{a}
SIG (mmol/L)	−8.6 (−15 to −4)^{ac}	−6.0 (−12 to −2.0)^{ab}	−10 (−17 to −5.0)^{c}	−7.0 (−12 to 0.5)^{b}	−5.0 (−10 to −0.6)^{b}
A_{tot} (mmol/L)	13 (11–14)^{a}	11 (10–12)^{b}	12 (10–13)^{b}	13 (12–15)^{c}	12 (11–13)^{a}

Data presented as median and Q25 and Q75 range. Different letters within a row indicate a statistically significant difference ($P < .05$).

TPP, total plasma proteins; HCO_3^-, bicarbonate; p_aCO_2, arterial partial carbon dioxide pressure; AG, anion gap; SID_m, measured strong ion difference; SIG, strong ion gap; A_{tot}, total plasma concentration of nonvolatile weak acids.

Table 2. Results of the forward stepwise regression of measured arterial pH as dependent variable versus physicochemical variables of 793 hospitalized foals.

Disease Group	Order of Entry into Regression Model	Variable	Partial r^2	Model r^2
Septic (n = 417)	1	SIG	20.2	20.2
	2	SID_m	28.1	48.3
	3	p_aCO_2	33	81.3
	4	A_{tot}	8.2	89.5
Nonseptic (n = 376)	1	SIG	26.3	26.3
	2	SID_m	32.6	58.9
	3	p_aCO_2	22	80.9
	4	A_{tot}	6.7	87.6
NE (n = 82)	1	SIG	38.2	38.2
	2	p_aCO_2	20.5	58.7
	3	SID_m	30.3	89
	4	A_{tot}	4.6	93.6
Prematurity (n = 38)	1	SIG	21	21
	2	p_aCO_2	25	46
	3	SID_m	40	86
	4	A_{tot}	2	88
Enterocolitis (n = 78)	1	SIG	16.1	16.1
	2	SID_m	44.2	60.3
	3	p_aCO_2	15.3	75.6
	4	A_{tot}	14.4	90

p_aCO_2, arterial carbon dioxide pressure; SID_m, strong ion difference; SIG, strong ion gap; A_{tot} total plasma concentration of nonvolatile weak acids; NE, neonatal encephalopathy.

P values were < .0001 for all variable coefficients.

Sensitivity and Specificity of Anion Gap and Strong Ion Gap to Predict Severe Hyper-L-lactatemia

The subset of 81 foals had a similar breed distribution and included 35 (42%) fillies and 46 (48%) colts with a median age of 1.6 days. Final clinical diagnoses included sepsis (35, 43%), pneumonia (29, 36%), enterocolitis (19, 24%), NE (15, 18%), prematurity/dysmaturity (9, 11%). The remaining 9 (11%) foals were diagnosed with a variety of other conditions, including neonatal isoerythrolysis, uroabdomen, and chyloabdomen. Admission values of the arterial blood gas, plasma concentration of electrolytes, total proteins, L-lactate, and AG and SIG of the 81 foals are presented in Table S3.

Calculated AG was significantly associated with plasma [L-lac$^-$] (Fig 1). Linear regression analysis generated the following equation: AG = (15.6 ± 1.2) + (1.42 ± 0.18) × [L-lac$^-$] (r^2 = 46%; $P < .0001$). The values in parentheses are estimates for the intercept or coefficient value ± SE of the estimate. The slope was significantly >1 and the intercept was significantly different from 0 ($P < .0001$). Calculated SIG was significantly associated with plasma [L-lac$^-$] (Fig 2). Linear regression analysis generated the following equation: SIG = (−4.7 ± 1.2) – (1.41 ± 0.17) × [L-lac$^-$] (r^2 = 45%; $P < .0001$).The slope was significantly smaller than −1 and the intercept was significantly different from 0 ($P < .0001$).

The areas under ROC analysis of AG and SIG for prediction of clinically important hyper-L-lactatemia were 0.89 (95% CI, 0.80–0.95; $P < .0001$), and 0.90 (95% CI, 0.81–0.96; $P < .0001$), respectively. The ROC analysis identified that AG > 27 mmol/L had a sensitivity of 80% (95% CI, 56–94; $P < .0001$) and specificity of 85% (95% CI, 73–93 $P < .0001$) and SIG < −15 mmol/L had a sensitivity of 90% (95% CI, 68–98; $P < .0001$) and specificity of 80% (95% CI, 68–90; $P < .0001$) to predict severe hyper-L-lactatemia. Comparison between area under ROC analysis of AG and SIG for predicting hyper-L-lactatemia did not show a significant difference ($P = .63$) (Fig 3).

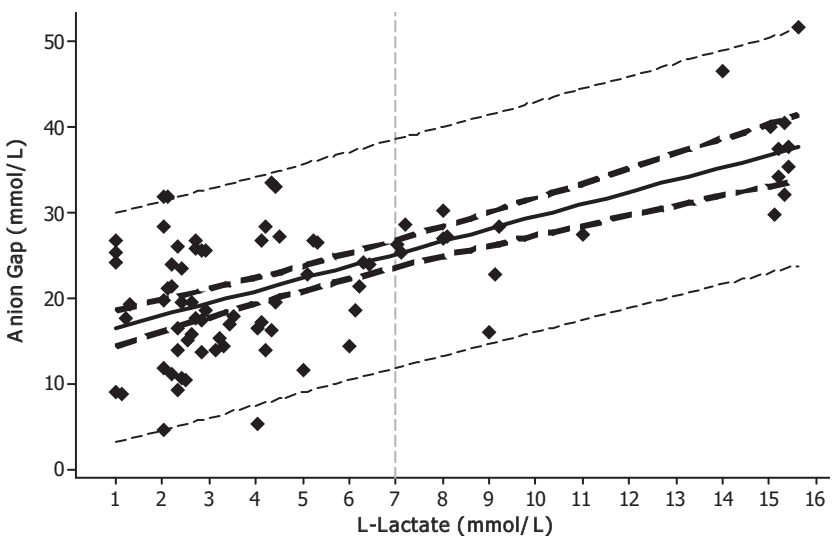

Fig 1. Scatterplot demonstrating the relationship between plasma concentration of L-lactate ([L-lac$^-$] and anion gap (AG) in hospitalized foals (n = 81). Each symbol represents values for 1 foal. The solid line is the line of best fit for linear regression analysis, and dotted lines represent 95% confidence intervals. Equation for line of best fit was as follows: AG = (15.6 ± 1.17) + (1.42 ± 0.17 × [L-lac$^-$]) (r^2 = 46%; $P < .0001$). The values in parentheses are estimates for the intercept or coefficient value ± SE of the estimate. The slope was significantly greater than 1 and the intercept was significantly different from 0 ($P < .0001$). Values represent mean SE for the estimate. Dashed vertical line represents the cut-off of for hyper-L-lactatemia ([L-lac$^-$] > 7 mmol/L).

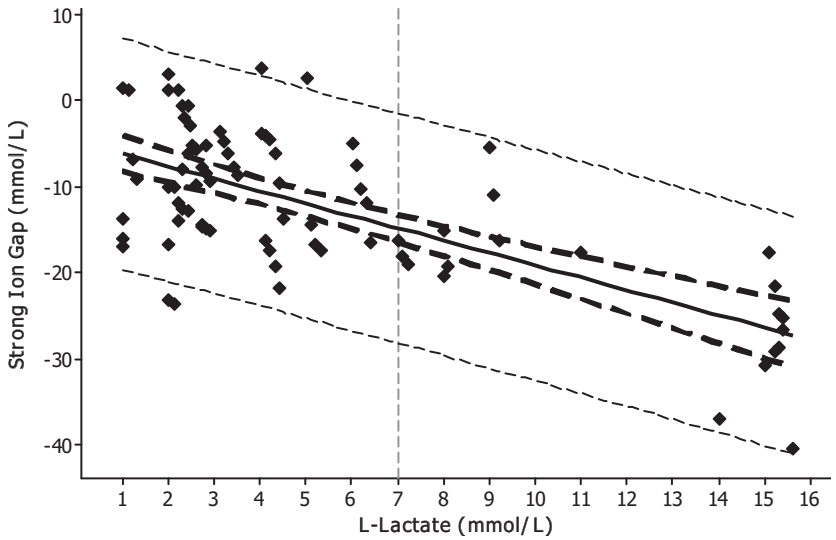

Fig 2. Scatterplot demonstrating the relationship between plasma concentration of L-lactate ([L-lac$^-$] and strong ion gap (SIG) in hospitalized foals (n = 81). Each symbol represents values for 1 foal. The solid line is the line of best fit for linear regression analysis, and dotted lines represent 95% confidence intervals. Equation for line of best fit was as follows SIG = (−4.7 ± 1.2) – (1.41 ± 0.17) × [L-lac$^-$] (r^2 = 45%; P < .0001). The values in parentheses are estimates for the intercept or coefficient value ± SE of the estimate. The slope was significantly smaller than −1 and the intercept was significantly different from 0 (P < .0001). Values represent mean SE for the estimate. Dashed vertical line represents the cut-off of for hyper-L-lactatemia ([L-lac$^-$] > 7 mmol/L).

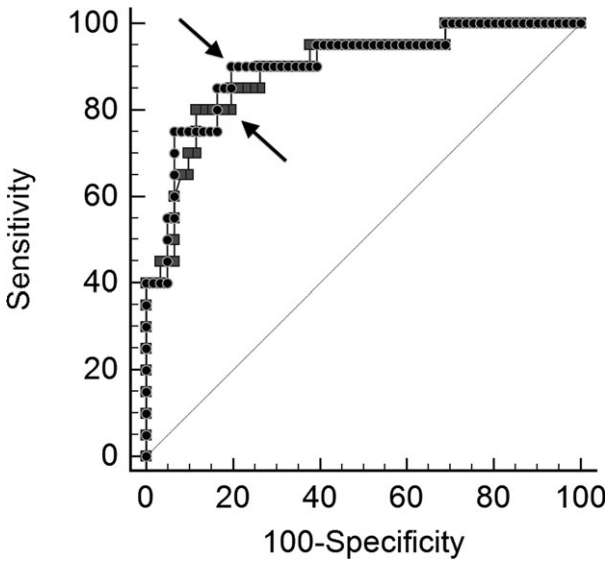

Fig 3. Receiver operator characteristic (ROC) curve analysis of anion gap (AG) and strong ion gap (SIG) for predicting hyperlactatemia ([L-lac$^-$] > 7 mmol/L) in 81 hospitalized foals with different clinical diagnosis. Area under the ROC for AG (squares) 0.89 (95% CI, 0.8–0.95) and SIG (dots) 0.90 (95% CI, 0.81–0.96). Arrows indicate the optimal cutpoint of AG (squares) and SIG (dots) for distinguishing hyperlactatemia.

Discussion

Use of the physicochemical approach indicated that acidemia was the most common acid–base disorder in hospitalized foals included in this study. Acid–base disorders were predominantly because of changes in the measured strong ion concentration (SID_m) and increase in the strong UAs concentration (increased SIG). Combined, the SID_m and SIG accounted for 54–68.5% of the changes in measured arterial pH. Similar results were reported previously in sick calves with or without diarrhea in which SID_m and SIG accounted for 58–75% of the changes of the calculated pH.[21,22]

The SID should be considered in the context of electrical neutrality. Certain elements such as Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- exist only as fully ionized entities in body fluids.[33] At physiological pH, this applies to anions such as sulfate, D-, and L-lactate$^-$, with pK_a values of 4 or less.[33] It is important to recognize that metabolic acidosis is produced by a decrease in the SID, which produces an electrochemical force resulting in alteration of plasma concentration of weak ions such as H^+, OH^-, HCO_3^-, CO_3^{2-}, and the anionic form of nonvolatile weak acids ($A\square^-$).[20] However, from the purely quantitative standpoint, HCO_3^- and A^- occupy the entire SID electrical space.[20] Other ions are present in such minute concentrations, measured in nmol/L, that they are quantitatively insignificant.[19,20]

The SID might be decreased by hyponatremia in presence of normochloremia or hyperchloremia, and hyper-L-lactatemia.[21,22] The high proportion of foals included in this study suffering from sepsis, accompanied by moderate to severe dehydration and increased concentration of plasma creatinine in nonsurviving foals might contribute to and explain the presence of increased [L-lac$^-$] and unmeasured strong ions resulting in SIG and AG acidosis.[34,35]

One should also note the magnitude of the AG and SIG in septic and nonsurviving foals. The mean AG

and SIG were 29, and −18 mmol/L, respectively (normal < 17 mmol/L and −2 mmol/L, respectively). Furthermore, the slope of the relationship between AG and [L-lac$^-$] (1.4), and SIG and [L-lac$^-$] (−1.4) were >1 and smaller than −1, respectively. These results strongly suggest that hyper-L-lactatemia is associated with increased concentrations of other UAs.

In this study [L-lac$^-$] only explained 45% of the AG and SIG concentration in this group of hospitalized foals. This represents a very large load of UAs different to L-lac$^-$.[36] The source of unmeasured anions remains to be determined. Several studies have shown that in human patients with hyper-L-lactatemia and "unexplained acidosis" with normal or near normal blood [L-lac$^-$], the plasma concentrations of acids associated with the tricarboxylic acid cycle are remarkably elevated.[37]

A study evaluating the concentration of pyruvate, D-lactate, acetoacetate, and 3-hydroxybutyrate concentration in adult horses with gastrointestinal diseases showed that pyruvate was increased but the concentrations were <10% of [L-lac$^-$]. The concentration of D-lactate was increased in half of the horses, with the highest concentration measuring 2.3 mmol/L.[38] It is possible that UAs other than L-lac$^-$ are present in high concentrations in neonatal hospitalized foals. However, this speculation remains to be proven. Other possibilities include increased negatively charged acute phase proteins,[36] or exogenous compounds in administered fluids (lactate, acetate, citrate, gluconate)[39] or medications (anion-containing β-lactam antibiotics, parenteral nutrition)[40] administered before hospital admission. However, because the retrospective design of this study we were unable to comment beyond this.

In this retrospective study, p_aCO_2 and A_{tot} had also significant contributions to plasma pH. Alterations in p_aCO_2 were important contributors, especially in those with prematurity or NE. Hypercapnia is common in each group because of decreased effective alveolar ventilation.[41,42] Weak acid (hypoproteinemic) alkalosis was a common acid–base disorder in this group of foals especially those with sepsis, prematurity, or NE. The role of plasma protein concentration in acid–base balance is well recognized.[43] The effect of the globulin concentration on plasma pH also requires consideration, as an altered albumin-to-globulin ratio could affect the effective values for $[A_{tot}]$[19] especially in foals with FTPI or sepsis or both. Although critically ill human and equine neonatal patients often present with hypoalbuminemia and hypogammaglobulinemia, there is no evidence that clinicians should treat hypoalbuminemia and hypoglobulinemia as an acid–base disorder. Currently, there is no evidence that the body regulates A_{tot} to maintain acid–base balance.[20]

The ROC curve analyses demonstrated that AG > 27 mmol/L and SIG <−16 mmol/L predict the presence or absence of clinically significant hyperlactatemia. Several studies have shown conflicting results regarding the sensitivity and specificity of AG when investigating metabolic acidosis.[1,5,25–27] In critically ill foals, a moderate correlation was demonstrated between AG and plasma [L-lac$^-$], with AG having a good sensitivity (77%) and specificity (83%) in detection of hyperlactatemia > 5 mmol/L.[1] In one study, AG was accurate and clinically useful for estimating UAs in horses with normal protein concentrations,[28] whereas, other studies have found that AG might be a poor parameter for prediction of mild hyperlactatemia due to the AG's susceptibility to fluctuations in albumin, pH, pCO_2, Ca^{2+}, Mg^{2+} and phosphate concentrations.[27,44,45] In this study, the AUC ROC analysis showed that calculated AG was a reliable surrogate for predicting clinically relevant hyper-L-lactatemia, regardless of the presence of hypoproteinemia. These findings are in agreement with a previous study in horses with acute diarrhea where AG was an excellent predictor of severe hyper-L-lactatemia ([L-lac$^-$] > 5 mmol/L) although 30% of the horses were hypoproteinemic and hypoalbuminemic.[29]

The reference range of calculated AG is wide and arises from contributions of 4 separate measured ions, each with their own reference range.[46] But, because we used a high [L-lac$^-$] cut-off (7 mmol/L) to define severe hyper-L-lactatemia, we guaranteed that the relative concentration of this unmeasured anion did not make it possible for the AG to remain within the reference range during a hyper-L-lactatemic state.[46] However, AG should be interpreted in the context of other pathophysiological disturbances in individual patients. As shown in this study, 32% of hospitalized foals are hypoproteinemic; this might result in a decreased AG that considerably underestimates the presence of unmeasured anions.[47] Unlike the AG, SIG treats total plasma protein and phosphate concentrations as variables and is not affected by changes in pCO_2 or pH.[17,28,44] In critically ill foals and calves, SIG correlated moderately with [L-lac$^-$] and was highly correlated with AG,[1,26] whereas Constable et al.[28] showed a strong correlation between SIG and plasma [L-lac$^-$] in healthy horses. Similarly, a previous study of horses with diarrhea showed that SIG and AG had a very good sensitivity and specificity to predict important hyper-L-hyperlactatemia (L-lac$^-$ > 5 mmol/L) in horses with acute diarrhea.[29] In this study, the AUC ROC analysis revealed that when the threshold of plasma [L-lac$^-$] was 7 mmol/L, the sensitivity and the specificity of SIG to predict hyper-L-lactatemia were excellent.

This study had several limitations, most notably its retrospective design, to the likely bias toward sicker patients. However, such foals seem to be representative of the population presented to referral teaching hospitals. Also, the number of subjects was small for each clinical diagnosis. Finally, one potential limitation of this study was the change in instrumentation from 1982 to 2007, particularly related to the use of ion selective potentiometry and its impact on the measured value for electrolyte concentration.[48] A recent study evaluated the impact of 2 different methodologies (indirect potentiometry and direct potentiometry) to measure strong ions concentrations on acid–base balance of critically ill horses. This study concluded that the ability to accurately predict blood pH of critically ill horses is depen-

dent on the methodology used to quantitatively measure strong ions especially Na^+, Cl^-, L-lactate$^-$, D-lactate$^-$, uremic anions.[48] Unfortunately, we were not able to determine the specific timing of instrumentation change in our teaching hospital and, therefore, comparison between methodologies could not be made.

In conclusion, the results of this study demonstrated that acid–base disorders in this group of hospitalized foals were predominantly because of an increase in the SIG, changes in SID_m and p_aCO_2 AG and SIG are good predictors of clinical significant hyper-L-lactatemia. The ROC curves of AG and SIG were almost identical. Therefore, AG and SIG could be used as surrogate tests for detection of hyper-L-lactatemia in hospitalized foals when direct measurement of [L-lac$^-$] is unavailable.

Footnotes

[a] Microsoft Access 2003; Microsoft Corp, Redmond, WA.
[b] Minitab Software, Phyladelphia, PA.

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Table S1. Admission values of plasma creatinine, electrolytes, and acid–base variables of 793 septic and nonseptic hospitalized foals.

Table S2. Admission values of plasma creatinine, electrolytes and acid–base variables of 793 survivors and nonsurvivors hospitalized foals.