Supplementary Material

Unmeasured Strong Anions as Prognostic Biomarkers of Metabolic Acidosis in Congolese Children with Severe Malaria: A Prospective Cohort Study

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I. Operational Definitions

a) **Severe Malaria according to the WHO criteria**:

Severe Malaria (SM) is defined as a composite of any Plasmodium parasitemia (falciparum or other species), detected by asexual stages on a peripheral blood slide or a positive rapid diagnostic test in combination with one or more of the following clinical and/or laboratory features, including:

- Impaired consciousness defined by a Blantyre coma score of <3,
- Multiple convulsions, defined by ≥2 seizures within a period of 24 hours,
- Prostration, defined by the inability to sit, stand, or walk without assistance,
- Clinically significant bleeding documented by the presence of recurrent or prolonged bleeding from the nose, gums, or venipuncture sites, hematemesis or melena,
- Acidosis, defined by a standard base deficit of >8 meq/l, a plasma bicarbonate level of <15 mmol/l, or a venous plasma lactate level of ≥5 mmol/L,
- Anemia, defined by a hemoglobin level of ≤5 g/dl or a hematocrit of ≤15%, combined with a parasite density of >10,000/µl,
- Hypoglycemia, defined by a plasma or serum glucose level of <40 mg/dl (<2.2 mmol/l),
- Parasitemia density of 10%,
- Jaundice, defined by a plasma or serum bilirubin level of >50 µmol/l (3 mg/dl) combined with a parasite density of >100,000/µl,
- Renal impairment, defined by a plasma or serum creatinine level of >3 mg/dl or a blood urea level of >20 mmol/l,

- Pulmonary edema or Respiratory insufficiency, defined by a confirmed edema on radiologic examination or an oxygen saturation of <92% while breathing ambient air with a respiratory rate of >30 breaths/min,
- Shock or circulatory collapse documented by a systolic blood pressure (SBP) of <70 mm Hg, combined with evidence of impaired perfusion (cool extremities or prolonged capillary refill)[1, 2].

b) **Respiratory distress** during severe malaria is defined by the WHO as deep breathing with intercostal retractions in the lower part of the rib cage without signs of localization in the thoracic region, suggesting metabolic acidosis.

c) **Other terms:**

- Normal pH: 7.4 ± 0.02
- Mild acidosis: any pH = 7.37–7.35
- Moderate acidosis: any pH < 7.35
- Severe acidosis: any pH < 7.2
- Alkalosis: any pH > 7.45
- Severe alkalosis: any pH > 7.6
- Normal PaO_2: 85 (+/- 5) mmHg or 11 pKa
- Normal $PaCO_2$: 40 (+/- 4) mmHg or 5.3 (+/- 0.5) pKa
- Cation: any ion carrying a positive charge.
- Anion: any ion carrying a negative charge.

- Strong anion (strong acid): any anion with a dissociation constant (pKa) in aqueous solution less than 4.5. It is considered completely dissociated. Lactate has a pKa of 3.9, and is therefore considered a strong anion.
- A weak anion (weak acid) is any anion with a pKa close to physiological pH. In aqueous solution, it exists in as many dissociated as undissociated forms. As such, they act as buffers capable of accepting an excess of protons. Bicarbonate has a dissociation constant of 6.8 and is the body's main extracellular buffer.

II. Prognostic Metabolic Acidosis Biomarker Calculation

To estimate the serum level of unmeasured acids, we calculated the standard base deficit, the anion gap and the strong ion gap.

a) Standard Base Deficit was calculated using the modified Van Slyke equation and corrected for differences in plasma albumin and phosphate using correction factors developed by Wooten[3, 4]. Albumin is expressed in g/dL and phosphate in mg/dL.

- SBDCAP = ($[HCO_3^-]$-24.4) + (8.3 *[Albumin]*0.15 + 0.29 * [Phosphate]*0.32) * (pH-7.4)

b) Anion gap was estimated and corrected for the negative charge of albumin and phosphate. Albumin is expressed in g/dL and phosphate in mg/dL.

- AG =([Na] + [K]) – ([Cl] + $[HCO_3]$)
- AGCAP = ($[Na^+]$ + $[K^+]$) - ($[Cl^-]$ + $[HCO_3^-]$) - (2 * [Albumin] + 0.5 * [Phosphate])
- AGCAPL = ([Na] + [K]) – ([Cl] + $[HCO_3]$) -(2 x albumin g/dL + 0.5 x phosphate mg/dL) - [lactate mmol/L]]
- AGCAPL = calculated AG - (2 x albumin g/dL + 0.5 x phosphate mg/dL) - [lactate mmol/L]]
- AGCAPL = AGCAP - Lactate(mmol/L)
- Phosphate (mEq/L) = Phosphore (mEq/L) x (0,309 x (pH - 0,469))
- Phosphate (mmol/L) = Phosphore (mmol/L) x (0,309 x (pH - 0,469))
- Phosphate (mg/dL) = (Phosphate (mmol/L) x 31) x 0,1
- From mmol en mg: Value in mmol x atomic (or molecular) weight = value in mg

- Atomic (or molecular) weight for Phosphore = 31
- 1L = 10 dL
- Albuminate (g/L) = albumine (g/L) x (0,123 x (pH-0,632))

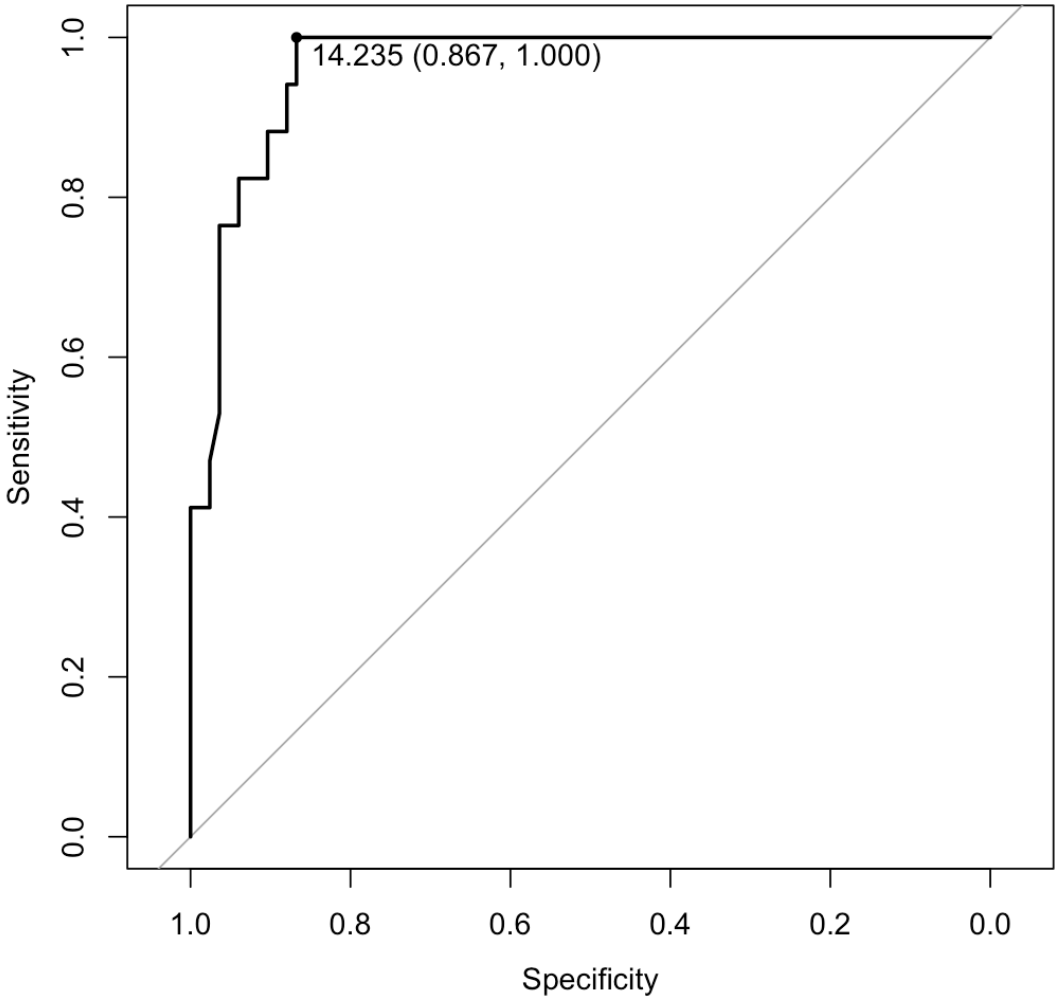
c) To calculate the strong ion gap, we used Stewart's Approach modified by Figge et al[5, 6]. and determined the apparent strong ion difference (SIDa) and the effective strong ion difference (SIDe), as follows:

- SIDa = ($[Na^+]$ + $[K^+]$ + $[Mg^{2+}]$ + $[Ca^{2+}]$) - ($[Cl^-]$ + [Lactate$^-$])
- SIDe = (2.46 * 10^$^{-8}$ * pCO_2 / 10^$^{-pH}$) + ([Albumin] * (0.123 * pH – 0.631)) + ($[PO^{4-}]$ * (0.309 * pH – 0.469)))

Finally, the strong ion gap (SIG) was calculated by subtracting the apparent and effective strong ion differences:

- SIG = SIDa – SIDe

III. Supplementary Figures



Supplementary Figure 1(SF1): The optimal SIG cutoff value, which was identified using the Youden index derived from standard logistic regression-based ROC analysis. A SIG value ≥ 14.235 was associated with a high risk of in-hospital mortality, whereas a SIG value < 14.235 was associated with a lower risk of death. At this threshold, elevated SIG demonstrated excellent prognostic performance, with a Youden index of 0.84, a sensitivity of 100%, and a specificity of 86.7%, indicating a strong association between higher SIG levels and increased in-hospital mortality.

IV. Supplementary Tables

Supplementary Table 1 (ST1): Firth Univariable Logistic Regression

Variable	β (SE)	OR (95% CI)	*P*-value	Method
SIG	1.01 (0.26)	2.74 (1.79 - 5.32)	$9.63e^{-13}$	2
Method: 1-Wald, 2-Profile penalized log-likelihood, 3-None Likelihood ratio test=50.91735 on 1 df, p=9.633405e-13, n=100 Wald test = 16.79937 on 1 df, p = 4.154709e-05				

Abbreviations: SIG, Strong Ion Gap; SE, Standard Error; OR, Odds ratio

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