Evaluation of pKa as a cause of discordance between calculated and measured bicarbonate in arterial and venous blood

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Abstract

**Background:** The measurement of bicarbonate level in blood is extremely common and often provides vitally important data used in the care of critically ill patients. The bicarbonate level in blood can be directly measured or derived from calculations using the Henderson-Hasselbalch equation; mostly adopted by the blood gas analyzers. Arterial blood gas (ABG) analysis is commonly performed for clinical evaluation, but the procedure has certain limitations in the form of reduced patient acceptability (because the procedure can be painful) and the potential to cause complications such as arterial injury, thrombosis with distal ischemia, hemorrhage, aneurysm formation, median nerve damage and, rarely, reflex sympathetic dystrophy.

**The aim of the study:** If there is discordance between arterial and venous blood gas parameters including pH, pCO₂, bicarbonate, Sodium, Potassium, chloride and discordance between measured and calculated bicarbonate in both arterial and venous blood samples.

**Materials and methods:** Comparison study involving 250 patients for whom clinical Judgment was made that arterial blood sample is needed for assessment of acid-base status. Both arterial and venous blood samples were collected using heparinized autosampler syringes PICO 50 as close in time as possible and were analyzed in Arterial Blood Gas analyzer ABL 80 flex.

**Results:** There was a statistically significant difference between arterial and venous pO₂ (126±48.5 vs 62±30.5, p= 0.001) and SO₂ (95% vs 68%, p=0.03).

**Conclusion:** According to the study results traditionally measured venous bicarbonate can be a

A convenient substitute for calculated arterial bicarbonate in critically ill ICU patients. However, more accurate assessments will require ABG for additional parameters. Besides, the present study design did not involve the collection of data on patient demographics, the severity of illness, and a requirement for inotropic support or prognosis.

**Key words**
Arterial Blood Gas, Measured Bicarbonate, Calculated Bicarbonate, Henderson Hasselbach Equation.

**Introduction**
Arterial Blood Gas analysis (ABG) is crucial in managing metabolic and respiratory acid-base disorders and in assessing oxygenation in critically ill patients. The three parameters essential for such decisions are pH, the partial pressure of carbon dioxide pCO₂ and Bicarbonate concentration [HCO₃⁻]. The Bicarbonate value obtained in an arterial blood gas analyzer is a calculated parameter [1]. The calculation is based on the fact that the major form of transport of carbon dioxide in the blood is in the form of bicarbonate [2].

According to Henderson – Hasselbach equation [1],
\[ \text{pH} = \text{pKa} + \log \left( \frac{[\text{Base}]}{[\text{Acid}]} \right) \]

In the equilibrium reaction of carbon dioxide, carbon dioxide is the acid and bicarbonate is the base. pKa of carbonic acid is 6.1. Hence the Henderson Hasselbach equation is modified as,
\[ \text{pH} = 6.1 + \log \left( \frac{[\text{HCO}_3^-]}{[\text{CO}_2]} \right) \]

As carbon dioxide concentration is proportionate to the partial pressure of carbon dioxide by a proportionality constant, solubility coefficients [3],
\[ [\text{CO}_2] = s \times p\text{CO}_2 \]
The solubility coefficient of carbon dioxide or Bunsen coefficient is approximately 0.03 mmol/L per mmHg at 37°C. On further simplification of equation 1, we get a formula to calculate bicarbonate concentration.

\[ \text{HCO}_3^- = 0.03 \times p\text{CO}_2 \text{ Antilog (pH - pK')} \]

However, this calculation is based on the assumption that both pKa (6.1) and solubility coefficient (0.03) are constants value, which is not true. pKa of bicarbonate is increased in acidic pH and is decreased in alkalotic pH [4]. pKa is also affected by temperature variations [5]. These variations are observed more in acutely ill patients, as they present with extremes of pH and temperature [6]. Solubility coefficient is also affected by pH and ionizing strength of blood. This might result in underestimation and overestimation of Bicarbonate concentration in extremes of pH which may mislead us in both diagnosing the primary acid-base disorder and analyzing the magnitude of compensation. Bicarbonate concentration, on the other hand, can be measured directly in both arterial and venous blood with acceptable discordance by enzymatic method. This measurement is not affected by changes in pH. In addition, direct measurement of Bicarbonate by enzymatic method measures total carbon dioxide (TCO₂) or the potential bicarbonate [7]. Total Carbon dioxide is a sum of dissolved carbon dioxide, bicarbonate, carbonic acid, carbonate, and carbamates. Knowledge of total carbon dioxide is essential in deciding the requirement and dosage of bicarbonate therapy in acidosis. However, measured bicarbonate is affected by the presence of organic acids like acetylsalicylic acid, valproic acid, benzoic acid. All these acids cause an overestimation of measured bicarbonate [8]. Calculated Bicarbonate [HCO₃⁻] obtained in Arterial-Blood-Gas (ABG) analyzer is affected by pH and ionizing strength of blood. Direct measurement of Bicarbonate, which measures total carbon dioxide (TCO₂) is not affected by these changes and is essential in deciding on bicarbonate therapy. Hence, a discordance between measured and calculated bicarbonate is expected [9].
Materials and methods
The study was conducted in the year 2017 in Omandurar Govt. Estate, Chennai. Totally 250 patients for whom clinical Judgement was made that arterial blood sample was needed for assessment of acid-base status. Both arterial and venous blood samples were collected using heparinized autosampler syringes PICO 50 as close in time as possible and were analyzed in Arterial Blood Gas analyzer ABL 80 flex. The plasma from both arterial and venous blood samples was separated by centrifugation at 37°C and was utilized to measure bicarbonate by the enzymatic method developed by Forrester, et al. [6]. The method was based on phosphoenolpyruvate method. We used a fully automated clinical chemistry analyzer for this purpose.

Results
There was no statistically significant difference between arterial and venous pH (7.382±0.436 vs 7.367±0.456, p= 0.31), pCO₂ (39.3±14.32 vs 41.9±16.11, p=0.23), calculated HCO₃⁻ (22.91±7.93vs 24.15±8.21, p=0.24), measured bicarbonate (24.82±8.15vs25.63±9.21, p=0.43), anion gap (11.21±3.74 vs 10.78±4.89, p=0.22). There was a statistically significant difference between arterial and venous pO₂ (126±48.5 vs 62±30.5, p= 0.001) and SO₂ (95% vs 68%, p=0.03). Bland Altman analysis revealed narrow limits of agreement when arterial and venous pH, pCO₂, calculated Bicarbonate, measured bicarbonate and anion gap values (0.04 to 0.11, -0.3 to -3.4, -1.1 to -3.8, -2.1 to -4.4, 2.1 to -1.1 respectively). Bland Altman analysis revealed narrow limits of agreement when arterial and venous pH, pCO₂, calculated Bicarbonate, measured bicarbonate and anion gap values (0.04 to 0.11, -0.3 to -3.4, -1.1 to -3.8, -2.1 to -4.4, 2.1 to -1.1 respectively). On Bland Altman analysis, poor agreement in PO₂ and SO₂ measurements between arterial and venous samples were observed. 95% limits of agreement for PO₂ and SO₂ include 145.3 to 32.9 and 33 to 12. There was a statistically significant difference when arterial measured and calculated bicarbonate values were compared (24.82±8.15 vs 22.91±7.93). There was a weak correlation between pH and bias in TCO₂ and [HCO₃⁻] in arterial (r=0.576, p=0.01) and venous samples (r=0.532, p=0.01) as per Table 1.

Table - 1: Comparison of various parameters in arterial and venous blood samples.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameter</th>
<th>Arterial Value</th>
<th>Venous Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>pH</td>
<td>7.382</td>
<td>0.218</td>
<td>7.367</td>
</tr>
<tr>
<td>2</td>
<td>pCO₂</td>
<td>39.3</td>
<td>7.16</td>
<td>41.9</td>
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<tr>
<td>3</td>
<td>Calculated Bicarbonate</td>
<td>22.91</td>
<td>3.97</td>
<td>24.15</td>
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<tr>
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<td>Measured Bicarbonate</td>
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<td>4.08</td>
<td>25.63</td>
</tr>
<tr>
<td>5</td>
<td>Anion Gap</td>
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<td>1.87</td>
<td>10.78</td>
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<tr>
<td>6</td>
<td>PO₂</td>
<td>126</td>
<td>24.25</td>
<td>62</td>
</tr>
</tbody>
</table>

Discussion
Arterial Blood gas analysis is essential in managing critically ill patients, who often present with extremes of pH and temperature. Many medical decisions are based on the bicarbonate value of the arterial blood gas analysis report [10]. For example, the anion gap calculation, which is essential for identifying the type of metabolic acidosis is dependent on the bicarbonate value. However, the calculated bicarbonate value in an ABG report is found to be affected by many variables. Hence, we proposed that measured bicarbonate in a simultaneously collected venous sample should be included in the panel of blood gas reports [11]. The agreement between arterial and venous pH, pCO₂, TCO₂ and [HCO₃⁻] and discordance between arterial and venous pO₂ indicate that venous blood sample would suffice for
identifying and managing an acid-base disorder unless oxygen delivery is to be assessed [12]. The wider LOA of both arterial and venous total carbon dioxide or in other words measured bicarbonate and calculated bicarbonate indicate that TCO2 has to be measured to assess the acid-base status [13]. However, the weak correlation between pH and bias between measured and calculated bicarbonate indicate that predicting measured bicarbonate based on pH and calculated bicarbonate values is not possible. At the same time, we should have in mind the possibility of measured bicarbonate getting affected by the presence of organic acids, as organic acids are well-known interferences for measured bicarbonate estimation by enzymatic method [14].

**Conclusion**

The measurement of bicarbonate level in blood is extremely common and often provides vitally important data used in the care of critically ill patients. The bicarbonate level in blood can be directly measured or derived from calculations using the Henderson-Hasselbalch equation; mostly adopted by the blood gas analyzers [1]. Arterial blood gas (ABG) analysis is commonly performed for clinical evaluation, but the procedure has certain limitations in the form of reduced patient acceptability (because the procedure can be painful) and the potential to cause complications such as arterial injury, thrombosis with distal ischemia.

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**References**

11. Severinghaus JW, Stupfel M, Bradley AF. Variations of serum carbonic acid

