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Acid-Base and Electrolyte Teaching Case

Use of Anion Gap in the Evaluation of a Patient With Metabolic Acidosis

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High anion gap (AG) metabolic acidosis, a common laboratory abnormality encountered in clinical practice, frequently is due to accumulation of organic acids such as lactic acid, keto acids, alcohol metabolites, and reduced kidney function. The cause of high AG metabolic acidosis often is established easily using historical and simple laboratory data. Despite this, several challenges in the diagnosis and management of high AG metabolic acidosis remain, including quantifying the increase in AG, understanding the relationship between changes in AG and serum bicarbonate level, and identifying the cause of high AG metabolic acidosis when common causes are ruled out. The present case was selected to highlight the importance of the correction of AG for serum albumin level, the use of actual baseline AG rather than mean normal AG, the relationship between changes in serum bicarbonate level and AG, and a systematic diagnostic approach to uncommon causes of high AG metabolic acidosis, such as 5-oxoproline acidosis (pyroglutamic acidosis).
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INDEX WORDS: 5-Oxoproline acidosis; pyroglutamic acidosis; high anion gap metabolic acidosis; acetaminophen; glutathione; cysteine; glutamate–cysteine ligase.

Note from Feature Editor Jeffrey A. Kraut, MD: This article is part of a series of invited case discussions highlighting either the diagnosis or treatment of acid-base and electrolyte disorders. Advisory Board member Glenn T. Nagami, MD, served as the Consulting Editor for this case.

INTRODUCTION

Anion gap (AG), representing the difference in concentration between measured cations and anions in serum, commonly is used for assessment of the accuracy of laboratory data and analysis of acid-base disorders. It primarily consists of the concentration of serum albumin, as well as phosphate, sulfate, and organic acids. Interpretation of AG therefore requires correction for serum albumin concentration. A wide variation in normal values can be observed within and between clinical laboratories; therefore, knowledge of the laboratory's normal range is important. Moreover, when possible, actual baseline AG for the patient being evaluated should be used.[1]

High AG metabolic acidosis is a common acid-base disorder encountered in clinical practice. When nonchloride acids accumulate in body fluids, the hydrogen ion is neutralized by bicarbonate and nonbicarbonate buffers in the extra- and intracellular compartments, whereas the conjugate base (or A^-) accumulates and AG increases. Accumulation of organic anions such as lactate, keto acids, Kreb cycle intermediates (citrate, isocitrate, α-ketoglutarate, and D-lactate), inorganic anions such as sulfate and phosphate, and exogenous substances (and their metabolic byproducts) such as formate and glycolate are the most frequent known causes of an increase in AG (Box 1).

The cause of high AG metabolic acidosis usually can be established through a thorough history, physical examination, and evaluation of easily available laboratory data. However, in some patients, specialized tests may be required to establish a clear diagnosis. The present teaching case describes our approach to a patient with high AG metabolic acidosis, highlighting important pitfalls in the use of this diagnostic tool.

CASE REPORT

Clinical History and Initial Laboratory Data

An 80-year-old woman is referred for evaluation of high AG metabolic acidosis in the setting of altered mental status and worsening diarrhea. Her history was notable for atrial fibrillation, hypothyroidism, Crohn disease without bowel resection, and a knee arthroplasty complicated by methicillin-susceptible *Staphylococcus aureus* bacteremia 1 month earlier. Her medications included amiodarone, 200 mg, daily; levothyroxine, 1.25 mg, daily; mesalamine, 1,000 mg, 3 times daily; pantoprazole, 40 mg, daily; and acetaminophen as needed for knee pain. On examination, she had a heart rate of 60-70 beats/min and blood pressure in the range of 110-130s/60-70s mm Hg. She was cachectic and arousable, but did not follow commands. Her mucus membranes were dry, neck veins were flat, and she had an irregularly irregular heart rate. The rest of her physical examination findings were unremarkable. Laboratory tests obtained at the time of admission

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Box 1. Causes of High Anion Gap Metabolic Acidosis

Common
- Lactic acidosis
- Ketoacidosis
- Acute kidney injury
- Chronic kidney disease
- Methanol poisoning
- Ethylene glycol poisoning
- Salicylate poisoning

Uncommon
- Diethylene glycol poisoning
- Propylene glycol poisoning
- 5-Oxoproline acidosis
- D-Lactic acidosis

showed the following values: sodium, 138 mEq/L; potassium, 4.7 mEq/L; chloride, 108 mEq/L; bicarbonate, 11.3 mEq/L; serum albumin, 1.6 g/dL; AG, 19 mEq/L; corrected AG, 25 mEq/L; serum glucose, 78 mg/dL; serum urea nitrogen, 30.0 mg/dL; and serum creatinine, 1.0 mg/dL with estimated glomerular filtration rate of 57 mL/min/1.73 m^2 calculated using the 4-variable MDRD (Modification of Diet in Renal Disease) Study equation. Urinalysis was negative for ketones. Historical review of laboratory data showed a prior AG of 10 mEq/L and albumin level of 4.0 g/dL.

Additional Investigations

Because the cited studies did not clarify the cause of the patient's high AG metabolic acidosis, the following studies were carried out and showed the following results: serum lactate, 13.5 mg/dL; serum salicylate, <5 mg/dL; serum acetoacetate, 0.412 mmol/L; β-hydroxybutyrate, 0.06 mmol/L; measured serum osmolality, 309 mOsm/kg; calculated serum osmolality, 291 mOsm/kg; urine creatinine, 38.5 mg/dL; and urine AG, 53.3 mEq/L. Alcohols and D-lactate were not measured. Her AG remained elevated for the first 3 days of hospitalization. Further history obtained from the patient's extended care facility revealed that she was taking acetaminophen, 650 mg, every 6 hours on a regular basis. 5-Oxoproline (pyroglutamic) acidosis was suspected, and urine study results in our genetic laboratory were strongly positive for 5-oxoproline.

Diagnosis

5-Oxoproline (pyroglutamic) acidosis related to acetaminophen use.

Clinical Follow-up

Acetaminophen treatment was discontinued and tramadol therapy was instituted for pain control. The patient was given 1 dose of *N*-acetylcysteine. The patient's mental status improved and AG and serum bicarbonate values returned close to their baselines over the course of 6 days (Fig 1).

DISCUSSION

Assessment of patients with AG metabolic acidosis should begin by identifying the normal range for AG in the laboratory conducting the analysis, correcting AG for serum albumin level and comparing the corrected AG with the baseline corrected anion gap (Box 2). The correction factor is a 2.5-mEq/L increase for each 1-g/dL decrease in serum albumin level.[2] Although this patient's AG was increased at 19 mEq/L, corrected AG was 25 mEq/L. It is important to note that if AG were not corrected for serum albumin level, the increase in her AG of 9 mEq/L would be significantly less than the decrease in her serum bicarbonate level of 14 mEq/L, raising the possibility of mixed AG and non-AG metabolic acidosis. If corrected AG is used, the increase in AG and decrease in serum bicarbonate level would be similar, supporting the diagnosis of a simple high AG metabolic acidosis. Also, the increase in AG and decrease in serum bicarbonate level will not always be equivalent. The nature of the accumulating anion, time after onset, and level of kidney function will affect this relationship and should be taken into consideration.[3] It

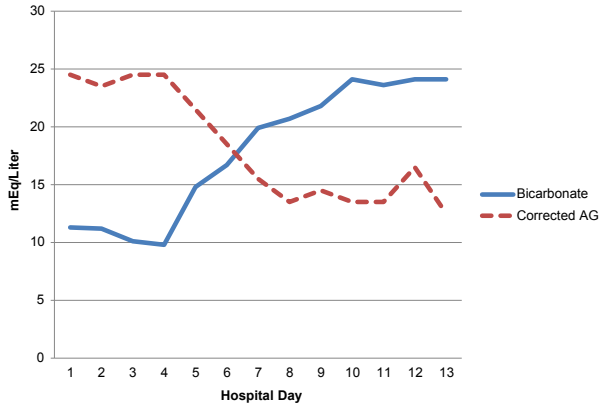

Figure 1. Serum bicarbonate concentration and corrected anion gap (AG) over the 13 days of hospitalization.

Box 2. Algorithm for Evaluating High Anion Gap Metabolic Acidosis

Step 1: Identify the normal range for the laboratory performing the analysis
Step 2: Correct the anion gap for serum albumin level
Step 3: Compare the corrected anion gap to the patient's baseline anion gap
Step 4: Evaluate the patient's past & present kidney function (as measured by creatinine and estimated glomerular filtration rate) to determine the kidneys' ability to actively manage and clear an acid load
Step 5: Evaluate the osmolal gap
Step 6: Order serum alcohols (methanol, ethylene glycol, propylene glycol) if the osmolal gap is elevated or if it is "normal" and there is suspicion of alcohol ingestion
Step 7: Order serum lactate if there is suspicion of end-organ hypoperfusion or a history of metformin or propylene glycol use
Step 8: Order serum keto acids if there is suspicion of malnutrition, starvation, or uncontrolled diabetes
Step 9: Order serum salicylate if aspirin use is known or suspected
Step 10: Order D-lactate if there is a history of bowel resection, short bowel syndrome, or active inflammatory bowel disease
Step 11: Order serum or urine 5-oxoproline if there is a history of malnutrition and acetaminophen use

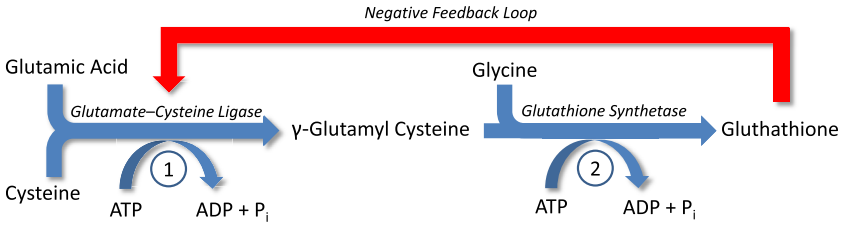

Figure 2. Glutamate–cysteine ligase catalyzes the production of the dipeptide γ-glutamyl cysteine from glutamic acid, cysteine, and the hydrolysis of adenosine triphosphate (ATP). Glutathione synthetase then catalyzes the production of glutathione from γ-glutamyl cysteine, glycine, and the hydrolysis of ATP. Excess glutathione inhibits glutamate–cysteine ligase. Abbreviations: ADP, adenosine diphosphate; P_i, inorganic phosphate. Source: Emmett.[6]

is more frequent to observe a 1:1 relationship between these parameters with ketoacidosis than lactic acidosis. The relationship in oxoproline acidosis remains undefined. Given this complexity, the anion of interest, such as lactate, should be measured directly when possible because the change in AG does not always reflect its actual concentration.[1]

Our patient presented with a marked increase in AG and the initial workup quickly ruled out the common causes of high AG metabolic acidosis, including reduced kidney function, diabetic ketoacidosis, and lactic acidosis. Her osmolal gap was mildly elevated to 19 mOsm/kg, raising the possibility of exogenous toxins. In coming from a supervised setting, this possibility was considered very unlikely in this patient. As a rule, in patients with unexplained high AG metabolic acidosis, an elevated osmolal gap should trigger a workup to exclude exogenous toxins such as methanol and ethylene glycol, and less commonly, diethylene glycol and propylene glycol. However, it should be noted that the normal osmolal gap does not rule out toxicity in a patient with an elevated AG. This is especially true in patients presenting long after ingestion when the parent compound is mostly converted to toxic metabolities.[4] Poisoning by diethylene glycol, a compound used in cosmetics and as a coolant, can present with neurologic symptoms and acute kidney injury. Propylene glycol, a common solvent in cosmetics and intravenous medications, is metabolized to lactic acid. These two possibilities were excluded quickly using historical data. Two other possibilities needed to be considered: D-lactic acidosis and 5-oxoproline acidosis. The former is seen only with short bowel syndrome, blind loops of bowel, fistulas, and large diverticuli. It is due to bacterial fermentation of carbohydrates and presents with ataxia, dysarthria, altered mental status, and coma. Although this patient had Crohn disease, this condition was in remission. In addition, she had not undergone bowel resection, which made this diagnosis unlikely. Given the history of acetaminophen intake, the possibility of 5-oxoproline acidosis was raised and the diagnosis was established by its measurement.

Since the first case of 5-oxoproline acidosis was reported in 1989, the number of case reports and reviews has increased exponentially.[5,6] In a thorough review of this condition, Emmett[6] reported that most cases (40 of 49) involve women with chronic medical diseases, malnutrition, reduced kidney function, and long-term acetaminophen use. This constellation is not uncommon, and the low incidence of 5-oxoproline acidosis may reflect both the exclusion of 5-oxoprolinemia from the differential and the relative paucity of facilities with assays that can detect 5-oxoproline in serum or urine.

5-Oxoproline is a cyclic form of glutamic acid. The pathophysiology of 5-oxoproline acidosis can be understood best through a discussion of the factors that affect glutathione production and elimination. Glutathione is produced in 2 adenosine triphosphate (ATP)-dependent reactions that unite glutamic acid, cysteine, and glycine into a tripeptide. Glutathione serves multiple functions: it is a storehouse for cysteine, regulator of the cell cycle, transcellular transporter of amino acids, and disposal pathway for reactive species.[6-8] When a sufficient amount of glutathione is produced, glutamate-cysteine ligase (GCL) is inhibited by a negative-feedback mechanism.[9] This process is illustrated in Fig 2. Glutathione also is a substrate for 5-oxoproline production. In this pathway, the removal and transfer of other amino acids from glutathione allows glutamic acid to autocyclize and become 5-oxoproline, as illustrated in Fig 3.[10] The accumulation of 5-oxoproline can be linked directly to depletion of cysteine stores, a rate-limiting substrate required for the production of glutathione, or depletion of glutathione stores by acetaminophen metabolites. Because cysteine

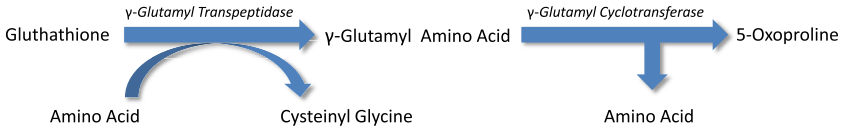

Figure 3. Gamma-glutamyl transpeptidase catalyzes the production of γ-glutamyl amino acid from glutathione and an amino acid; γ-glutamyl cyclotransferase catalyzes the production of 5-oxoproline and an amino acid from γ-glutamyl amino acid. Source: Meister.[10]

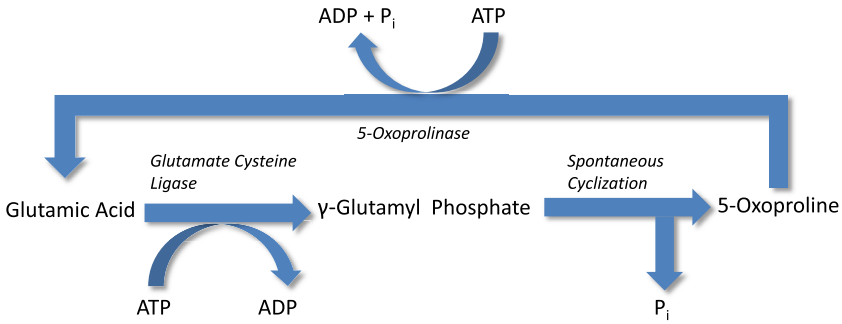

Figure 4. Glutamate–cysteine ligase first catalyzes the production of γ-glutamyl phosphate with hydrolysis of adenosine triphosphate (ATP). In the absence of a cysteine residue, it undergoes spontaneous cyclization with loss of a phosphate and forms 5-oxoproline. 5-Oxoproline then is converted back to glutamic acid by 5-oxoprolinase and hydrolysis of ATP. Abbreviations: ADP, adenosine diphosphate; P_i, inorganic phosphate. Source: Emmett.[6]

availability is dictated by diet, methionine metabolism, the trans-sulfuration pathway, and γ-glutamyl transpeptidase expression, conditions that limit the availability of cysteine, can result in reduced glutathione levels.[8] With decreased glutathione concentrations, GCL activity remains uninhibited. Uninhibited GCL phosphorylates glutamic acid, which then spontaneously cyclizes into 5-oxoproline in the absence of cysteine.[6,11] This process can be seen in Fig 4.

When acetaminophen is introduced in the setting of cysteine depletion, glutathione stores are depleted even further. Normally, 90% of a single dose of acetaminophen is metabolized into nontoxic metabolites by glucuronidation or sulfation.[12,13] Around 5% of the acetaminophen that remains is metabolized by cytochrome P450 2E1 into the highly reactive electrophilic intermediate *N*-acetyl-*p*-benzoquinoneimine.[12,13] Glutathione stabilizes the reactive *N*-acetyl-*p*-benzoquinoneimine by conjugation. Glutathione stores therefore can be depleted if acetaminophen ingestion is excessive. The combination of glutathione depletion and uninhibited GCL activity then leads to 5-oxoproline acidosis when cysteine is not available. 5-Oxoproline ultimately can be converted back to glutamic acid by 5-oxoprolinase and hydrolysis of ATP.[14] Enzymatic activity of 5-oxoprolinase has been shown in rat and bovine models, but has yet to be defined in humans.[15,16]

The classic presentation of 5-oxoproline acidosis is high AG metabolic acidosis in a frail elderly woman ingesting acetaminophen. In our case, the explanation for 5-oxoproline acidosis was clear: deconditioning, poor nutrition, and chronic diarrhea following a prolonged hospital stay that resulted in cysteine depletion and was exacerbated by regular dosing of high-dose acetaminophen. With improved nutrition and cessation of acetaminophen treatment, the high AG metabolic acidosis resolved and returned close to baseline over the course of the following week. *N*-Acetylcysteine has been used previously to increase cysteine availability and glutathione concentrations in the setting of hereditary glutathione synthetase deficiency.[17] Given the potential benefit of *N*-acetylcysteine coupled with its low side-effect profile, pursuit of this therapy is reasonable until future studies provide evidence to the contrary.[18]

One finding that needs further comment is the positive urinary AG of 53.3 mEq/L. In metabolic acidosis, one would expect a normal kidney to compensate by increasing ammonium production to allow the excretion of additional hydrogen ions. Urinary AG, an indirect measurement of urinary ammonium, therefore should be markedly negative and not positive.[19] However, this holds true only if there are no abnormal organic anions in the urine. Although it has not been well studied in this disorder, we suspect that the positive urinary AG is due to the presence of a high level of organic anions, including 5-oxoproline, in urine. This finding has been well documented in 2 case reports.[20,21]

The diagnosis of high AG metabolic acidosis requires a logical approach by initially correcting AG for changes in serum albumin level, quantifying the degree of change in AG by comparing it with the baseline AG and then using other data to establish the most common causes, including accumulation of lactic or keto acids, retention of endogenous acid due to reduced kidney function, or production of acid from metabolisms of a variety of alcohols (Box 3). If the initial workup does not provide a specific diagnosis, one should consider less common causes, such as D-lactic or 5-oxoproline acidosis. Although unexplained high AG metabolic acidosis often triggers a series of specialized testing not routinely available, this decision should be driven by attention to historical and clinical data.

Box 3. Teaching Points

1. To use anion gap effectively, know the mean and range of normal anion gap values in your laboratory, correct the anion gap for albumin, and when possible, compare the anion gap with your patient's baseline anion gap.
2. The patient's history and other laboratory values should always be used to guide the choice of tests when considering potential causes of high anion gap metabolic acidosis.
3. If common diagnoses are ruled out, consider less common disorders, such as 5-oxoproline acidosis, which occur in the setting of acetaminophen use in malnourished patients, particularly with cysteine deficiency.

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