Laboratory Anomalies in the Basic Metabolic Panel: Core Curriculum 2025

Layana T. Takieddine and Joe M. El-Khoury

Laboratory testing plays an integral part in medical decision making. However, laboratory results can sometimes vary significantly, leading to anomalous outcomes that are not consistent with the clinical picture. These anomalies can occur even in the best of laboratories simply because the total testing process includes elements that are not totally under the laboratory's control. For example, variations in patient preparation and sample collection procedures, as can happen at physician offices or patients receiving intravenous fluids, are major contributors to these anomalies. Therefore, physicians must remain aware of the causes of these anomalies so they can consider them when interpreting laboratory results and help implement solutions to mitigate them at their respective institutions. This Core Curriculum examines several instances where an understanding of preanalytical, analytical, and postanalytical variation is essential for detecting anomalies and providing proper patient care.

Complete author and article information provided at end of article.

Correspondence to J.M. El-Khoury (joe.el-khoury@yale.edu)

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The Core Curriculum aims to give trainees in nephrology a strong knowledge base in core topics in the specialty by providing an overview of the topic and citing key references, including the foundational literature that led to current clinical approaches.

Introduction

Laboratory data play a crucial role in shaping medical decisions: an estimated 70% of medical decisions are based on laboratory results. Therefore, timely and accurate laboratory data are essential for correct diagnosis, prognosis, and effective disease treatment. However, the total testing process, which begins when a test is ordered and ends with result interpretation by the ordering provider, comprises a number of important steps. Concerningly, some of these steps, such as the preanalytical steps, are often outside the laboratory's control and have a higher likelihood of error (Fig 1). Laboratory errors contribute to additional testing, heightened expenses, patient distress, and, in severe cases, potential patient morbidity or mortality.

So providers interpreting laboratory results have to remain vigilant when they come across anomalies—results that are not consistent with the clinical presentation. Anomalies can sometimes arise due to errors that occur in the total testing process but also may result from limitations of the testing methodology employed (eg, immunoassay interferences). Instead of simply labeling these as "lab error," we encourage providers who encounter similar laboratory anomalies to connect with their laboratory professionals to discuss these issues and implement solutions or raise awareness about them.

Laboratories have several tools at their disposal to help detect, reduce, and/or eliminate such anomalies. Unfortunately, many laboratories may not have the right expertise in place to address them. This installment of *AJKD*'s Core Curriculum in Nephrology will examine several instances where an understanding of preanalytical, analytical, and postanalytical errors is essential for detecting anomalies and providing proper patient care.

Serum Sodium

Hyponatremia, defined as a serum sodium (S_{Na}) concentration of ≤135 mmol/L, can be classified into 3 categories, each with different underlying causes:

1. Hypotonic hyponatremia is seen in conditions of increased water retention.
2. Hypertonic hyponatremia is seen in the presence of high osmolar solutes.
3. Isotonic hyponatremia is the case where pseudohyponatremia should be suspected.

Pseudohyponatremia is a laboratory artifact that can result from an analytical interference primarily affecting high-throughput chemistry analyzers with an indirect ion-selective electrode (ISE). Symptomatic hyponatremia is associated with nausea, headache, vomiting, and possible complications of seizures; when hyponatremia is asymptomatic, pseudohyponatremia should be ruled out before initiating treatment. Inappropriately treating false low sodium concentrations using fluid restriction and hypertonic saline may cause severe dehydration and hypernatremia. Patients with hyperlipidemia or hyperproteinemia are particularly susceptible to misdiagnosis with hyponatremia due to the nature of the laboratory test for sodium concentrations.

Although this may seem like semantics, it is important to note that hyperglycemia also causes hyponatremia, but it should not be called pseudohyponatremia. In this case, the

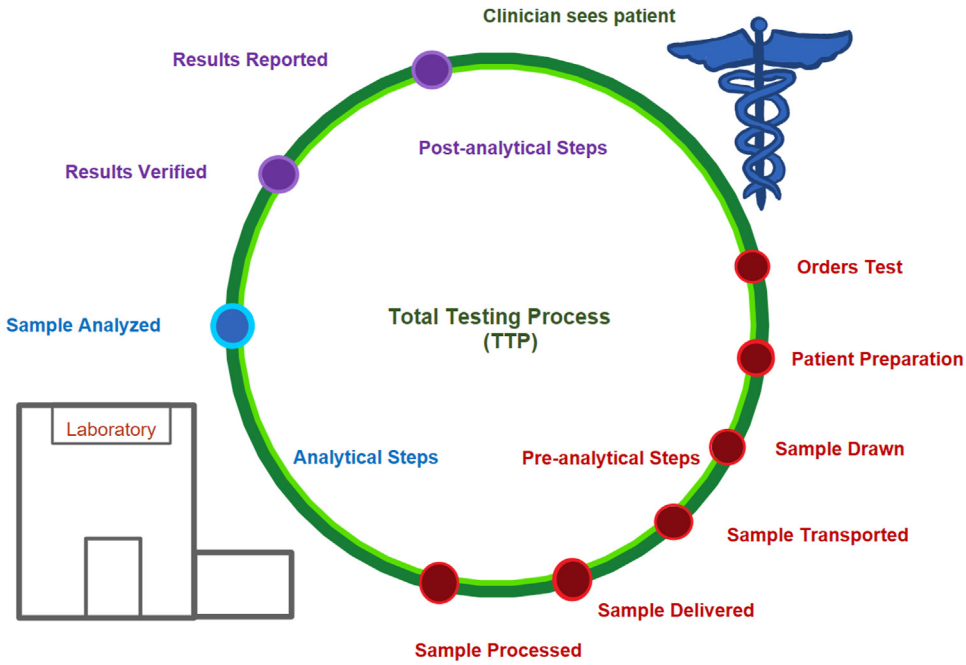

Figure 1. The total testing process (TTP). TTP is divided into 3 phases: preanalytical, analytical, and postanalytical steps. The preanalytical phase begins with the physician ordering the test after seeing the patient, and it includes all the steps up to the point of getting the patient sample tested on the analytical instruments (the analytical step). Everything that happens to the result after it is generated by the instruments in the analytical step and up to reporting it to the provider is referred to as the postanalytical steps.

S_{Na} is physiologically, not analytically, lower due to the presence of very high concentrations of glucose. So in hyperglycemic hyponatremia S_{Na} is truly lower, but the low sodium should not be treated because it will automatically resolve on administration of insulin to lower glucose. This is why we will not discuss hyperglycemic hyponatremia any further in this case.

Ion-selective electrode (ISE), the method currently used by most laboratories in the world to measure S_{Na} concentrations, measures the ionic activity of a solute and equates it to the solute concentration in the serum or blood sample. There are 2 types of ISEs: (1) indirect and (2) direct. In indirect ISE, the sample is diluted significantly before the immersion of the electrode in solution, and S_{Na} is measured with the assumption that serum is composed of 93% water and 7% proteins and lipids (Fig 2). In direct ISE the sample does not require dilution before analyses, and the S_{Na} is unaffected by changes in serum water/protein/lipids percentage concentration because the electrode directly measures the sodium concentration in whatever water fraction is available (Fig 2). Therefore, although indirect ISE has been the preferred method for automated chemistry analyzers, because dilution helps extend the life of the electrodes and lowers the volume of the sample needed for testing, it is obviously not ideal for some patients. As illustrated in Figure 2, the presence of high amounts of proteins or lipids expands the nonaqueous phase of the serum, which leads to a relative decrease in the aqueous phase where serum sodium is partitioned. For these patients, measurement of serum sodium by direct ISE is preferred.

It is important to note that direct ISE measures serum sodium at around 154 mmol/L in healthy individuals, which is the same sodium concentration as prepared in normal saline (0.9% NaCl). However, a correction factor is applied to align the results from direct ISE methods with those of indirect ISE and flame photometry (the historic method), which is why blood and serum sodium by direct and indirect ISE methods average around 140 mmol/L in a healthy individual.

Case 1: *An 83-year-old woman with anuric chronic kidney disease (CKD; stage 5) was admitted for elective intravenous immunoglobulin (IVIG) treatment. Before her treatment, a basic metabolic panel was ordered, which showed a baseline S_{Na} concentration of 136 mmol/L (reference interval: 135-145 mmol/L), fasting plasma glucose of 113 mg/dL (reference: <100 mg/dL), and creatinine of 6.5 mg/dL (reference interval: 0.5-1.2 mg/dL). Then IVIG (2 g/kg; 6% at 45 g IV each day for 2 days) was administered to the patient. The tests after IVIG infusion showed her S_{Na} concentration had dropped to 126 mmol/L.*

Question 1: What caused this patient's serum sodium concentrations to drop from 136 to 126 mmol/L?

(a) Hyperproteinemia
(b) Hypertriglyceridemia
(c) Hyperglycemia
(d) Chronic kidney disease

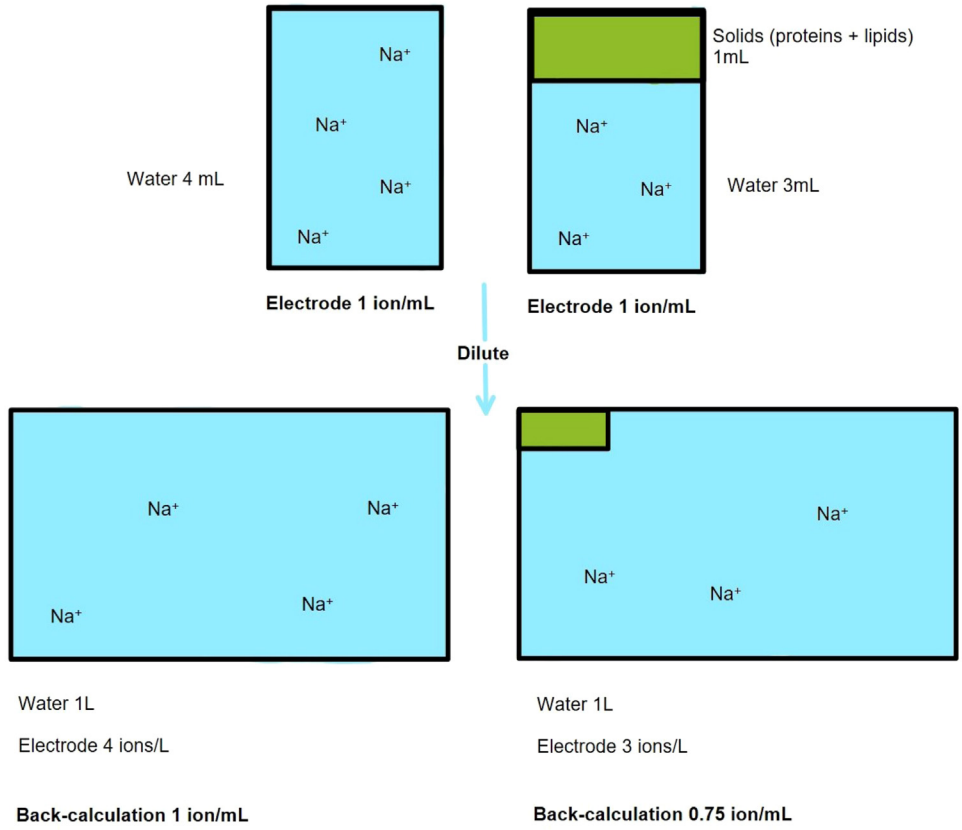

Figure 2. An illustration of the volume displacement effect that causes pseudohyponatremia for indirect ion-selective electrode methods. On the left side, a water solution without any proteins or lipids and with a sodium concentration of 1 ion/mL is significantly diluted to 1 L before an electrode is immersed to measure the concentration of sodium in water. The back-calculation to the 4 mL initial solution accurately also yields 1 ion/mL. While on the right side, a solution containing 25% lipids and proteins will inaccurately back-calculate to 0.75 mL/ion instead, because the total ions transferred are only 3 sodium ions due to the large amounts of lipids and proteins.

Question 2: What action should the clinician take next?

(a) Request the laboratory perform another sodium measurement by direct ISE.
(b) Request the laboratory spin down the sample to remove excess lipids then rerun sodium by same method.
(c) Treat the hyperglycemia with insulin and the hyponatremia will resolve by itself.
(d) Treat the patient's hyponatremia.

For the answers to these questions, see the following text.

Indirect ISE was used to measure this patient's S_{Na} concentration, which decreased from 136 mmol/L to 126 mmol/L after the patient had been given IVIG. Treatment with IVIG can significantly raise the total protein concentrations for patients, often to concentrations of >12 g/dL, which can cause artifactually lower sodium concentrations as measured by indirect ISE. Although we do not have enough information to know whether this patient had hypertriglyceridemia, we can rule it out as the cause here because the patient's baseline sodium was within the reference interval and hypertriglyceridemia does not spontaneously resolve. Although the patient does have elevated glucose concentrations at 113 mg/dL, this is not high enough to cause the deviation in sodium observed here. Therefore, the answer to Question 1 is (a), hyperproteinemia.

Recommendations to Confirm Pseudohyponatremia

When pseudohyponatremia is suspected, direct ISE methods such as point-of-care devices that typically measure sodium in whole blood are a preferred follow-up test (when available) because they are not affected by the volume displacement effect. Therefore, the answer to Question 2 is (a), request the laboratory perform another sodium measurement by direct ISE. Unfortunately, these methods are not compatible with large-scale automation, so not all patient samples can be run on these devices.

Almost all core laboratories are measuring serum sodium using indirect ISE methods, which are susceptible to interference from high proteins and lipids. However, laboratories can develop a procedure to detect and run samples that have significant concentrations of proteins or lipids by direct methods. Hyperlipidemia can be detected

by a lipemia index, which is already run by many laboratories on every sample going through the chemistry automation line to check for the presence of lipid interference. Total proteins are run on all comprehensive metabolic panels, thus detecting hyperproteinemia at least for some of those panels. Then cutoff points can be established that qualify a sample for direct ISE.

Some laboratories may already have such cutoffs for lipids and total proteins, but they may be inappropriately set. It is important for laboratories to note that most manufacturers set an allowable error of 10% for their interference studies, which is highly inappropriate for S_{Na}. For a patient with a sodium concentration of 140 mmol/L, 10% represents an allowable difference of ±14 mmol/L, so reporting down to 126 mmol/L would be considered acceptable, which is inappropriate. Reference change value calculations have shown that a change of ±4 mmol/L is highly significant for S_{Na}. For lipemia, recently published studies also have shown that current manufacturers are overstating the tolerance for interference because they define their lipemia thresholds by performing spiking experiments using Intralipid (a soybean oil, egg yolk phospholipids, glycerin, and water emulsion) instead of human samples containing high concentrations of endogenous lipids. It is important for practicing clinicians to know what their laboratory is doing to detect pseudohyponatremia and what its lipemia and hyperproteinemia cutoffs are based on (if they exist).

Pseudohyponatremia has gone unchecked for far too long because most laboratories and in vitro diagnostics manufacturers have used a far too lenient allowable error for acceptability for sodium when performing interference studies—10% instead of ±4 mmol/L—and they have inappropriately used Intralipid to evaluate the effect of lipemia on S_{Na} measurements by indirect methods. With appropriate cutoffs, laboratories can automatically detect and reflex samples with high lipids and proteins to be run on direct ISE methods, changing pseudohyponatremia from a prevalent laboratory artifact to a historical one.

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Serum Potassium

Hyperkalemia occurs when serum potassium is ≥5.5 mmol/L. This occurrence is common in patients with chronic kidney disease (CKD) and is aggravated by acute kidney injury (AKI), cardiovascular disease, diabetes mellitus, and medications that inhibit the renin-angiotensin-aldosterone system. However, it is not unusual to observe blood samples with falsely elevated potassium. The latter phenomenon, known as pseudohyperkalemia, requires proper assessment of a patient with hyperkalemia to rule it out (especially when no associated electrocardiogram changes are observed) and avoid unnecessary treatment and possible harm to the patient. For example, unnecessary administration of insulin to resolve pseudohyperkalemia could cause hypoglycemia and hypokalemia.

Common causes for pseudohyperkalemia include hemolysis of red blood cells during sample collection, mechanical stress during specimen handling, leukocytosis, thrombocytosis, and/or low temperature. However, colorimetric techniques can easily detect hemolysis (by measurement of free-floating hemoglobin in plasma) although the results from those samples may be withheld by today's laboratories. Hemolysis, especially at gross levels, is typically not a major cause for pseudohyperkalemia, if the laboratory's policies dictate withholding such erroneous results. Therefore, it is important that laboratories evaluate the impact of hemolysis (as detected spectrophotometrically by measuring free hemoglobin in serum, commonly called H-index) on potassium, and establish appropriate cutoffs for releasing results with comments when a sample is slightly hemolyzed (potassium changes around 0.3-0.7 mmol/L). In addition, a secondary cutoff should be established for when potassium results are canceled due to severe hemolysis (potassium changes greater than 0.7-1.0 mmol/L).

Because hemolysis remains a major cause of sample rejection, careful sample handling is essential to avoid any unnecessary mechanical stresses that can induce hemolysis and delay patient results, including those of potassium. Mechanical stress during sample processing includes vigorous mixing during collection as well as the pressurized centrifugal forces, that can occur via pneumatic tube transport. Leukocytosis or increased white blood cell counts seen in chronic lymphocytic leukemia can intensify cell membrane fragility, allowing potassium leakage into

the serum. Thrombocytosis also can lead to pseudohyperkalemia for blood collected in serum-producing tubes (ie, tubes that promote clotting, usually yellow-top tubes) because the elevated number of platelets during clotting will release a significant amount of potassium ions into the serum.

Unlike hemolysis, pseudohyperkalemia due to leukocytosis or thrombocytosis is not detected by the laboratory, although some have established concentration cutoffs for white cells and platelets beyond which potassium is unacceptable. Therefore, clinicians need to be aware that patients with extremely high white cells ($>50 \times 10^9$/L) and platelets ($>500 \times 10^9$/L, which affects blood collected in serum-producing tubes only) may exhibit pseudohyperkalemia. For patients with severe thrombocytosis, collecting blood samples in plasma-producing tubes (ie, tubes that prevent clotting by using anticoagulant additives such as lithium heparin, usually green-top tubes) is recommended to bypass pseudohyperkalemia.

In addition, another important cause of falsely elevated potassium is prolonged sample exposure to low temperatures, which can happen with whole blood samples stored in lockboxes outside in the winter. Potassium is actively transported by the Na^+/K^+-ATPase (adenosine triphosphatase sodium/potassium pump) which hydrolyses 1 molecule of adenosine triphosphate (ATP) for every 2 K^+ ions moved in and 3 Na^+ out of the cell. Erythrocytes contain this antiporter, which seems to have reduced affinity for ATP at low temperatures; therefore, potassium leaks out along its gradient into extracellular fluid (plasma or serum). Similarly, delayed processing of blood samples will cause an exhaustion of glucose to produce ATP, resulting in the failure of the Na^+/K^+ pump.

Case 2: *A 57-year-old woman presented to the emergency department in Philadelphia, Pennsylvania, for an elevated serum potassium of 6.1 mmol/L (reference interval: 3.5-5.0 mmol/L), which was discovered during a routine annual checkup. New samples collected in the emergency department revealed a normal serum potassium of 3.8 mmol/L. The physical examination and electrocardiography were without signs of hyperkalemia. Oddly, this is the patient's third presentation for an elevated potassium level found during a routine annual checkup in January, only to reveal normal concentrations when tested at the hospital. It is worth noting that all her previous samples with elevated potassium were free of hemolysis.*

Question 3: Given the above information, what is the most likely cause of this patient's pseudohyperkalemia?

(a) Hemolysis
(b) Temperature-dependent pseudohyperkalemia
(c) Familial pseudohyperkalemia
(d) Leukocytosis

For the answer to this question, see the following text.

Further investigation revealed that the blood samples drawn outside the hospital were collected at 9:00 AM and were delivered to the central laboratory by 12:00 PM; in addition, the collection at the outside clinic was done in lithium heparin gel tubes (light green tops), which were centrifuged upon arrival at the laboratory. The laboratory investigation was able to rule out hemolysis by reviewing the hemolysis index (a spectrophotometric measure of hemoglobin) of the samples; all were found to be within the acceptable range for potassium. Therefore, (a) hemolysis can be eliminated.

The outside temperature was typical for the month of January in Philadelphia, ranging between 1.1 °C and 6.1 °C. If the sample was stored as whole blood (ie, plasma or serum were not separated from cells by centrifugation), this low temperature could be leading to a cold-induced leakage of potassium from cells into plasma/serum. This commonly occurs with physician offices that do not process samples on-site and simply drop them off in uninsulated lockboxes outside of their offices for couriers to pick up. Couriers typically take over 2 hours to pick up samples, which is enough delay in the winter to cause leakage of potassium from the cells (∼18% increase by 2 hours when kept refrigerated). However, cold temperature exposure alone does not explain the significant increase we see for our patient in Case 2 (∼61% increase). Hence, (b) temperature is not the most likely cause of pseudohyperkalemia in our patient.

After the preanalytical considerations were ruled out, further genetic investigation revealed that this patient has variants in the *ABCB6* gene, which codes for an ATP-binding cassette transporter. As a result, answer (c) is correct: familial pseudohyperkalemia (FP) was diagnosed, a disease that leads to increased leakage of potassium from erythrocytes at temperatures lower than body temperature (37°C). This condition is an autosomal dominant trait caused by a missense variant in *ABCB6*; its frequency is 1:500 in the European population. Usually, patients affected by FP are asymptomatic, and their condition is discovered by accident. No further treatment is needed; because the concentration of potassium in these patients varies greatly when stored at low temperatures, they are not suitable candidates for blood donation.

This case also highlights an aspect of pseudohyperkalemia that is problematic for physicians whose offices are not adjacent to their laboratories or who lack sample-processing capabilities during the winter months. For practices affected by this limitation, there are 3 ways to address it and prevent harm to the patients:

1. Collect blood samples in gel-containing tubes and directly centrifuge them before transport to the central laboratory. Once the samples are spun, the gel acts as a barrier that separates cells from plasma/serum.

2. If samples cannot be centrifuged at the collection site or physician office, it is essential that the whole blood sample is stored at room temperature, in insulated lockboxes, or in lockboxes kept in heated areas in the winter before transport to the laboratory. Otherwise, potassium will massively leak from the cells, especially in patients with *ABCB6* gene variant. Keeping whole blood at room temperature will cause glucose to rapidly deteriorate (at ~10% per hour); if glucose results are also needed for your patients, a second blood sample should be collected in a separate fluoride oxalate tube (usually grey-top tubes).
3. If neither option 1 or 2 is possible, the only available alternative to get results that are anomaly-free for potassium and glucose is point-of-care testing at the clinic. This avoids the storage/transportation issues associated with options 1 and 2 but has its own limitations due to the poor precision and accuracy of these devices.

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Serum Bicarbonate

Once the body's finely regulated acid-base equilibrium is disrupted, a host of complications arise. Bicarbonate is a base that the body relies on for normal pH balance. Plasma bicarbonate travels through the body as carbon dioxide (CO_2) dissolved in the blood. Thus, the concentration of CO_2 in the blood is a reliable indicator of the acid-base equilibrium. The lungs and kidneys cooperate to maintain a normal blood pH by removing excess acid; hence, low CO_2 reflects metabolic acidosis, which can be due to AKI, sepsis, alcohol overdose, diabetic ketoacidosis, and diarrhea. The adult reference interval for plasma bicarbonate is 22-29 mmol/L.

Factitious hypobicarbonatemia occurs when the plasma bicarbonate measurement is falsely low, which can be due to lipid interference or has been observed in samples with prolonged exposure to air (CO_2 rapidly evaporates in underfilled tubes or tubes that were opened but took longer than usual to be analyzed).

Case 3: *A 59-year-old man with elevated triglycerides of 8,243 mg/dL (reference interval: ≤150 mg/dL) had an extremely low measured bicarbonate of 4 mmol/L (reference interval: 22-29 mmol/L) but did not have any symptoms of metabolic acidosis. The concerned clinician contacted the laboratory technologist to investigate the unusual result. The technologist reviewed the time between sample receipt and analysis, which was less than 30 minutes and is typical for the laboratory. He then pulled the sample and visually inspected to confirm that it was appropriately filled (the tube was around three-quarters full). In the meantime, the clinician requested that his team measure blood gases for the patient, which returned a normal pH, pCO_2, and bicarbonate of 23 mmol/L.*

Question 4: What can explain the initial low bicarbonate of 4 mmol/L measured in the laboratory?

(a) Delay in sample analysis
(b) Underfilling of sample accelerated the evaporation of CO_2
(c) Patient with asymptomatic metabolic acidosis
(d) Hypertriglyceridemia interference

Question 5: Why was the second measurement of bicarbonate on a blood gas sample normal and different from the initial result?

(a) Blood gas samples are run immediately, so no delay in testing.
(b) Sample collected for blood gas analysis was appropriately filled.
(c) Bicarbonate on blood gas analyzers is calculated, not measured.
(d) Blood gas samples are spun before analysis, removing hypertriglyceridemia interference.

For the answer to these questions, see the following text.

There are 2 methods that are commonly used to determine plasma bicarbonate concentrations. In the first method, bicarbonate is measured via automated

instruments and is a reflection of the total carbon dioxide (tCO_2) in the blood. Bicarbonate constitutes 95% of tCO_2, and carbonic acid constitutes 5%. Hence, tCO_2 is an accurate estimate of plasma bicarbonate, but some interferences affecting automated chemistry analyzers, such as hypertriglyceridemia, may lead to false results.

Total CO_2 can be measured by indirect ISE or by the spectrophotometric enzymatic method. In indirect ISE, triglycerides impact bicarbonate concentrations just like solids impacted sodium concentrations (in Case 1) through the volume displacement effect. High triglycerides will reduce the aqueous phase of the sample thus falsely lowering bicarbonate concentrations. In the spectrophotometric method, white light is passed through a solution, and certain wavelengths are absorbed based on the properties of the analyte. The presence of lipids leads to turbidity, which scatters the white light and can also interfere. Therefore, the answer to Question 4 is (d), hypertriglyceridemia interference.

When bicarbonate concentrations are suspiciously low in a patient who does not show clinical signs of low CO_2, point-of-care methods used for arterial blood gas (ABG) can be run to confirm. Point-of-care instruments used to measure pH and pCO_2 can also accurately calculate the bicarbonate based on the Henderson–Hasselbalch equation.

$$pH = 6.1 + \log \frac{[HCO_3^-]}{0.03 \times P_{CO2}}$$

Both pH and P_{CO2} are measured using a direct ISE analyzer.

Usually, serum bicarbonate concentrations measured by the tCO_2 and calculated by the ABG should be within 10% of each other. Any discrepancy between the 2 values should indicate the possibility of hypertriglyceridemia. Hence, for this patient, hypobicarbonatemia (low CO_2) was false and caused by triglyceride interference with the automated chemistry analyzers. It is recommended that patients with elevated triglycerides (>1,000 mg/dL) be tested for their bicarbonate using point-of-care methods to verify low CO_2 concentrations. Hence, the answer to Question 5 is (c), bicarbonate on blood gas analyzers is calculated, not measured.

Automated analyzers usually measure plasma CO_2 concentrations by the phosphoenolpyruvate (PEP) carboxylase reaction where PEP and CO_2 are converted to oxaloacetate and phosphate, and then reduced to malate with simultaneous nicotinamide adenine dinucleotide oxidation. This decreased absorbance is proportional to the CO_2 content in blood which can be disrupted by the presence of high triglycerides in the sample.

Delayed analysis of the blood sample and the use of underfilled tubes are other reasons behind falsely low bicarbonate concentration, but the severe changes observed in this patient would not be explained by a 30-minute delay and a three-quarters full tube.

When a small volume of blood is placed in a large test tube there is a lot of air space, which leads to the loss of CO_2 and subsequently falsely low bicarbonate. Falsely low CO_2 has been seen in infants because obtaining a sufficient volume of blood is challenging. It has been shown that a volume of blood less than 2 mL in a 4 mL tube results in a significant loss of CO_2. Similarly, delayed sample processing leads to the same effect. It has been noted that 30 minutes after sample collection 32% of CO_2 was lost in vitro (when 0.3 mL of blood was collected in a 4 mL tube). Thus, to bypass falsely low CO_2 it is important to

1. Use pediatric tubes when collecting blood from pediatric patients to minimize air space.
2. Fill tubes at least three-quarters of the way to avoid accelerated evaporation.
3. Analyze tubes within 1 hour of collection and do not allow CO_2 to be added on as a test to existing tubes that have already been opened.

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Serum Creatinine

Creatinine is one of the most measured analytes for the assessment of kidney function. Its serial monitoring is particularly useful in the evaluation of AKI, which is characterized by a rapid elevation in blood creatinine. Creatinine concentrations should be closely monitored in patients who present to the hospital with trauma, sepsis, hypotension, or oliguria as well as in those who have had surgery, especially cardiopulmonary bypass, because those patients will be at higher risk of developing AKI. If patients are receiving nephrotoxic medications (such as aminoglycosides or vancomycin), are receiving medications that block the renin-angiotensin-aldosterone system, or have advanced CKD, their risk of developing AKI is also higher.

Creatinine, like all biomarkers, is subject to preanalytical, analytical, and biological variability. These

poorly recognized sources of variation can also contribute to differences in serial results, which is why some guidelines advocate reporting reference change values (RCV). The RCV are values that represent significant or highly significant changes in the serial measurements of a biomarker, calculated by factoring in preanalytical, analytical, and biological variabilities.

As discussed in the introduction, preanalytical variability describes changes that can happen due to factors that affect the sample—from patient preparation to sample collection and transportation to the laboratory (Fig 1). This type of variability is usually preventable, and its risk can be mitigated by following proper patient preparation and sample collection and transportation procedures. This includes preventing contamination when drawing blood through the same line or above an intravenous (IV) infusion. This is vital because contamination could in many cases alter creatinine concentrations by dilution or interference.

Creatinine varies biologically due to several factors such as glomerular filtration rate, muscle mass, sex, age, ethnicity, and diet. Creatinine is produced from the catabolism of muscles, so muscle mass plays a significant role when analyzing creatinine concentrations. Physicians should be careful when interpreting results in patients who have very low muscle mass (anorexia or amputees) or very high muscle mass (obesity and weight lifters). Another factor affecting creatinine is a diet rich in red meats, which increases both serum creatinine and glomerular filtration rate. The biological variation of serum creatinine includes a drop to 0.25 mg/dL from birth until the first month of life then a linear increase with age until 20 years old. Later, serum creatinine remains constant with a mean of 0.9 mg/dL for men and 0.7 mg/dL for women. After the age of 70 years, serum creatinine will increase again in both males and females.

Analytical variability arises from the imprecision of the assay used to measure creatinine concentrations. There are 2 types of assays commonly used to measure serum creatinine: the Jaffe alkaline picrate and the enzymatic methods. Both rely on colorimetric detection. In the Jaffe method, the yellow-orange color resulting from the reaction of serum creatinine with picrate can be quantified. By contrast, the enzymatic method relies on enzymatic reactions, which can differ depending on the assay's manufacturer. A drawback in the Jaffe is that picrate is not fully specific to creatinine and can react with other components, called pseudo-chromogens, as well as interact with bilirubin and some specific drugs. Although less common, interferences can also affect enzymatic methods, especially dopamine, dobutamine, bilirubin, and proline (found in high concentrations in certain IgG therapies).

A comparison of both methods shows that analytical precision is better for the enzymatic assay than the Jaffe, particularly in samples with low to normal low concentrations as is the case in pediatric patients. Therefore, using enzymatic methods to measure serum creatinine reduces random errors and is the recommended method, especially for pediatric hospital laboratories. However, it is worth noting that Jaffe is still the most used method in laboratories today. As a practicing nephrologist, it is important to note which method your laboratory employs and to be aware of its limitations.

When monitoring creatinine variability, per the Association for Diagnostics and Laboratory Medicine Academy (formerly the American Association for Clinical Chemistry) AACC AKI 20/20 guideline, a change (RCV) ≥ 0.20 mg/dL or ≥20% (whichever is greater) is highly significant; hence, samples with creatinine changes that are <0.20 mg/dL when the baseline creatinine is <1 mg/dL or 20% when the baseline creatinine is ≥1 mg/dL are not highly significant.

Case 4: *A 73-year-old man with a history of ischemic cardiomyopathy presented with decompensated heart failure and AKI. He was admitted to the cardiac intensive care unit where he received dopamine via central line for assisted diuresis. His serum creatinine was tested using the enzymatic creatininase method with samples taken over 7 days. In Figure 3, the graph shows erratic serum creatinine concentrations that vary from 0.32 mg/dL to 1.78 mg/dL (reference interval: 0.50-1.20 mg/dL).*

Question 6: What may be the cause of the variation in creatinine results over 7 days for this patient?

(a) AKI
(b) CKD
(c) Resolving AKI
(d) Preanalytical error
(e) Analytical error

Question 7: What is this patient's most likely true creatinine value?

(a) 0.4 mg/dL
(b) 0.8 mg/dL
(c) 1.0 mg/dL
(d) 1.3 mg/dL
(e) 1.8 mg/dL

Question 8: How can this variability be avoided?

(a) Better fluid and drug management to avoid overtreating patient.
(b) Use of better creatinine methods by the laboratory.
(c) Use of better collection techniques by phlebotomy.
(d) It cannot be avoided.

For the answers to these questions, see the following text.

Dopamine is administered in the coronary care unit in cases of hypotension and low cardiac output because it increases the heartbeat rate and force of contractions.

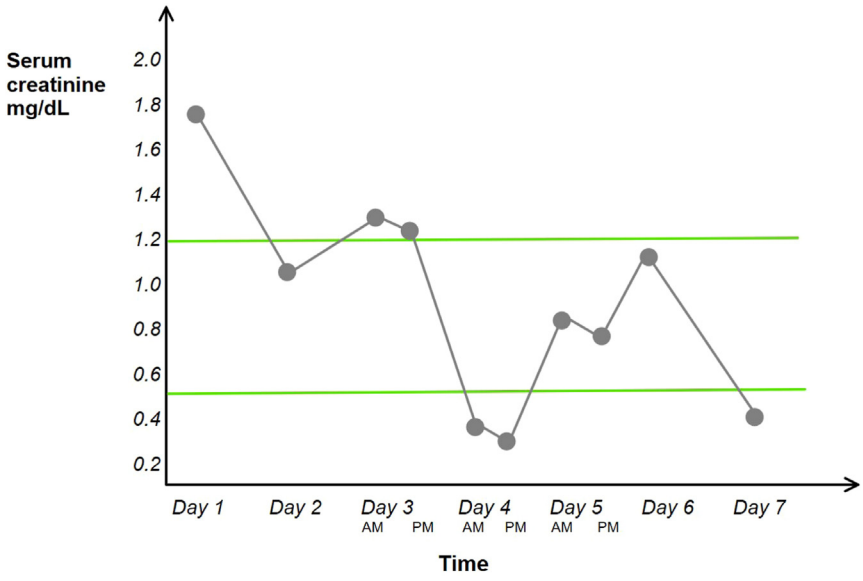

Figure 3. The graph shows the variation of serum creatinine (in mg/dL) as a function of days. The reference interval for serum creatinine is represented by 2 green horizonal lines at 0.5 mg/dL and 1.2 mg/dL, respectively. Over the 7 days the serum creatinine concentration varied erratically, with the highest concentration being 1.8 mg/dL and the lowest 0.32 mg/dL. Conversion factors for units: serum creatinine in mg/dL to μmol/L, ×88.4.

Unfortunately, dopamine interferes with the enzymatic creatininase assay by suppressing peroxidase, which uses phenazone as a substrate. Therefore, samples contaminated with dopamine will result in lower creatinine concentrations, which can mask a serious elevation if the results are within the normal range of 0.50-1.20 mg/dL. More specifically, the variation seen in the results for this patient is because the samples were inappropriately collected from the same line where dopamine is administered without appropriate line flushing and collection of a discard tube. The sample that was collected and sent to the laboratory for creatinine analysis was contaminated with dopamine administered through the same line. Variation in suppressed results can be explained by variation in the amount of dopamine contaminating each sample and falsely lowering the creatinine result to varying degrees. Therefore, the answer to Question 6 is (d), preanalytical error.

When the laboratory was contacted to explain this variability on day 7, it was recommended that a sample be drawn via a standard venipuncture from the arm opposite the IV to confirm. The creatinine result obtained on that sample was 1.8 mg/dL, confirming that all other results collected between day 1 and 7 had been affected by dopamine contamination due to improper collection from the IV line. Therefore, the answer to Question 7 is (e): 1.8 mg/dL.

This case sheds light on a poorly recognized preanalytical error, namely that creatinine could be subject to by dopamine interference with the assay. It is essential to recognize and avoid IV contamination because it leads to delayed diagnosis and wasted resources. Thus, the answer to Question 8 is (c), use of better collection techniques during phlebotomy.

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Pediatric Reference Intervals

Case 5: *A preterm female born at 30 weeks gestation with a birth weight of 1,300 g underwent surgery for a congenital heart defect at the age of 2 weeks. Her postoperative course was complicated by cardiogenic shock necessitating the*

administration of epinephrine. Subsequently, the neonate was closely monitored for AKI. To assess her renal function, creatinine and cystatin C concentrations were both ordered (as cystatin C measurement recently had become available at the hospital), revealing a normal creatinine concentration of 0.4 mg/dL (reference interval: 0.3-1.0 mg/dL) but an elevated cystatin C concentration of 2.7 mg/L (reference interval: 0.6-1.2 mg/dL).

Question 9: Which result is more consistent with the clinical picture?

(a) Creatinine
(b) Cystatin C

Question 10: How can the difference in results between creatinine and cystatin C be explained?

(a) Preanalytical: A problem with how the sample was collected (such as IV fluid contamination) or transported to the laboratory (such as pneumatic tube system transport) may have affected one or both test results.
(b) Analytical: A problem with an interference present in this patient's blood (such as epinephrine) may be affecting one or both test results.
(c) Postanalytical: A problem with how the results are reported and flagged may be involved (such as the reference intervals applied).

For the answers to these questions, see the following text.

Neonates are among the most susceptible patients to AKI, particularly those undergoing cardiac surgery. Their chance of AKI after cardiac surgery (CS-AKI) is 42%-64%. Preterm and low-birth-weight neonates are also at increased risk of AKI because 60% of nephron development happens during the third trimester of pregnancy and every additional kilogram in birth weight leads to 200,000 new nephrons.

Neonates born prematurely or at low birth weight are susceptible to low cardiac output after cardiac surgery. Low cardiac output syndrome is defined as a decrease in cardiac index to 2.0 L/min/m^2 at 6-18 hours after cardiac surgery. Undergoing cardiopulmonary bypass causes a systemic inflammatory response that leads to myocardial inflammation and a decline in ventricular performance.

Dopamine, dobutamine, epinephrine, and norepinephrine are often employed in preterm infants with hypotension and/or low cardiac output. However, it is important to note that although epinephrine can lead to a faster increase in heart rate than dopamine, it may result in hyperlactatemia and hyperglycemia. Therefore, epinephrine might not be the inotrope of choice in infants prone to metabolic complications.

The common biomarker for assessing kidney injury is serum creatinine; however, in recent years several shortcomings have been associated with the use of creatinine in neonates and small children as well as those with atypical muscle mass. First, creatinine does not change until 25% to 50% of the neonate's renal function is compromised, and its rise can only be detected after surgery in 36-48 hours. Hence, relying on creatinine can result in early-stage AKI going unnoticed and undiagnosed. Second, creatinine is a metabolic product of muscle breakdown so its production rate varies with muscle mass. Third (as mentioned in Case 4), serum creatinine measured by enzymatic methods can be negatively interfered with by catecholamines, causing the results to appear falsely lower than they actually are. However, the effect is more pronounced with dopamine and dobutamine, especially when a sample is collected via IV line. Epinephrine, which was administered to this neonate, may theoretically interfere, but it does not commonly have the pronounced effects of dopamine interference because it does not adhere to indwelling catheters as effectively. So its effects on this patient's creatinine results were likely minimal, if any (<5% variation).

In light of the limitations of serum creatinine discussed previously, other kidney function biomarkers can be used such as cystatin C, which has been growing in popularity in recent years due to its superiority at estimating kidney function when combined with creatinine in new race-free eGFR equations. Clinical laboratories are starting to offer this test routinely at their hospitals because the test is no longer considered esoteric; it is now available on common automated chemistry analyzers available in most hospitals.

Cystatin C is a low-molecular-weight protein produced at a constant rate by all nucleated cells. It can freely pass through the glomerulus and is independent of muscle mass, race, or gender. In addition, elevated concentrations of cystatin C generally reflect a decline in kidney function. However, neonates naturally have a much higher concentration of cystatin C, which can be misleading if physicians are unaware of the expected values of this biomarker in neonates. On average, cystatin C is around 1.78 mg/L during the first 6 to 30 days of life then it decreases to 1.25 mg/L at 3-5 months. The drop continues to 1.04 mg/L at 7-9 months then it reaches 0.96 mg/L at 12-14 months. A Canadian pediatric reference interval study, Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), established the reference intervals for patients younger than 1 month of age and showed that cystatin C ranges from 1.49 mg/L to 2.85 mg/L in healthy neonates. In this case, the reference interval of 0.6-1.2 mg/dL used to flag the patient's results by the laboratory was an adult range provided by the assay manufacturer and implemented by the laboratory.

Unfortunately, many assay manufacturers provide reference intervals only for adults (even after receiving clearance from the US Food and Drug Administration). As a result, many clinical laboratories may not have pediatric reference intervals available, and they are flagging results based on the adult intervals provided by the manufacturer. This can be very misleading for some tests. It is important that the laboratory employs pediatric reference intervals and that its result flagging is based on these.

In the absence of a national pediatric reference interval study in the United States, laboratories and providers are encouraged to adopt the CALIPER ranges that are appropriate to their instrument and assay. We would encourage pediatric nephrologists to communicate with their laboratory and consider how the pediatric reference intervals are established at their institution. Otherwise, pediatric nephrologists may be misinterpreting results because of inappropriate reference intervals or result flagging.

The good news for the patient in Case 5 is that both results are within the reference interval when appropriate pediatric reference intervals were applied. Therefore, the answer to Question 9 could have been both creatinine and cystatin C if the right reference intervals were applied. However, because the creatinine measure not only used the right reference interval but also provides an interpretation consistent with the clinical picture, the answer is (a) here.

However, it is important to note that cystatin C is quickly becoming the preferred test to assess kidney function for neonates and pediatric populations because it does not change with muscle mass. The new KDIGO 2024 guidelines recommend its use in the pediatric population as well.

The answer for Question 10 is (c), postanalytical: a problem with how the results are reported and flagged (such as the reference intervals applied).

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Conclusions

We hope that this installment has highlighted useful cases featuring issues that may go unnoticed in daily clinical practice. Our goal was to shed light on a few preanalytical, analytical, and postanalytical errors during the total testing process that may go undetected by the laboratory and may be released to the patient chart. This is why it is important for the practicing nephrologist to be familiar with where errors in the total process can arise so that they can identify them and work with their laboratory professionals to figure out solutions that will prevent them from happening again, where possible.

Article Information

Authors' Full Names and Academic Degrees: Layana T. Takieddine and Joe M. El-Khoury, PhD.

Authors' Affiliations: Medical Research Volunteer Program, American University of Beirut, Beirut, Lebanon (LTT); and Department of Laboratory Medicine, School of Medicine, Yale University, New Haven, Connecticut (JME).

Address for Correspondence: Joe M. El-Khoury, PhD, Department of Laboratory Medicine, 20 York St, PS535, New Haven, CT, 06510. Email: joe.el-khoury@yale.edu

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