Ionized Calcium: Its Significance and Clinical Usefulness*

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ABSTRACT

Maintenance of normal blood levels of ionized calcium (Ca\(^{2+}\)) plays an important role in the management of the critically ill patient. Therefore, Ca\(^{2+}\) should be collected properly and measured reliably. The sound analytical performance of today's Ca\(^{2+}\) analyzers using ion-selective electrode technology have made measurements accurate and precise. The introduction of this technology allows rapid and direct analysis in whole blood or serum, resulting in an enhanced reporting time. Since the amount of heparin in the syringe was shown to lower plasma ionized calcium concentration artifically, samples for plasma Ca\(^{2+}\) determination should be anticoagulated with a measured quantity of heparin. Ancillary factors in Ca\(^{2+}\) determination include effects of changes in sample pH, and situations where abnormal concentrations of calcium ligands are present. Many clinical situations require Ca\(^{2+}\) rather than total calcium measurements. Liver transplantation, citrated blood transfusions, and neonatal hypocalcemia are examples of a few such circumstances where determination of Ca\(^{2+}\) may be more physiologically and clinically meaningful than total calcium.

Introduction

The measurement of total calcium (Ca\(_T\)) concentration in serum is firmly established as a routine test in clinical chemistry. Since the advent of automated chemistry methods, it has been inexpensive, rapid, and easy to perform total calcium on large numbers of specimens which have required few special precautions in handling. In contrast, ionized calcium (Ca\(^{2+}\)) measurement has in the past been difficult, requiring relatively expensive, slow, and unreliable equipment and requiring special precautions in specimen handling. In recent years, however, there have been major improvements in Ca\(^{2+}\) analyzers which are now inexpensive, rapid (up to 40 samples can be analyzed per hour) and robust. At many institutions, ionized calcium in whole blood, plasma, or serum is routinely performed using direct poten-
The analytical performance of Ca\textsuperscript{2+} is at least as good as that of total calcium methods and far superior to the combined imprecision and inaccuracy of total calcium and albumin assays and the application of a nomogram for calculating Ca\textsuperscript{2+}.\textsuperscript{6}

It is also recognized that total serum calcium (Ca\textsubscript{T}) can be a poor indicator of the physiological state of calcium in a number of situations, particularly when abnormal concentrations of calcium ligands (e.g., lactate, citrate, bicarbonate) are present.\textsuperscript{11} The necessity for measuring ionized calcium (Ca\textsuperscript{2+}) in these situations is clear. This article will review the clinical utility for measuring Ca\textsuperscript{2+}.

**Distribution of Calcium in Blood**

The total calcium of plasma is the sum of the concentrations of many forms of calcium, including ionized calcium, calcium bound to albumin, and calcium bound to low molecular weight ligands as phosphate, bicarbonate, lactate, citrate, and others. The approximate distribution of calcium fractions in blood plasma is shown in figure 1. Together, the ionized and the ligand bound (complexed) calcium fractions are referred to as ultrafiltrable or dialyzable calcium. Protein-bound calcium is defined as calcium ions associated with proteins of MW 5000 or higher. The concentration of protein-bound calcium is directly proportional to the albumin concentration and is extremely pH dependent, since hydrogen ion competes with Ca\textsuperscript{2+} for binding sites on albumin. In some circumstances, exogenous anions, such as citrate and bicarbonate, may bind substantial additional amounts of calcium.\textsuperscript{12}

Since the distribution of calcium between all of these fractions is subject to the law of mass action, this equilibrium is primarily dependent upon calcium concentration and the concentrations of plasma proteins and low molecular weight ligand anions. Other factors

which also play a role in maintaining this equilibrium are pH, temperature, ionic strength, and magnesium ion concentration. Because of the marked dependence of Ca\(^{2+}\) on pH, these two parameters are usually measured concurrently. The measured Ca\(^{2+}\) can also be corrected or normalized to physiological pH of 7.4.\(^{17}\)

In healthy individuals the concentration of ionized calcium is directly correlated with the concentration of total calcium (Ca\(_T\)) because these individuals maintain fairly constant concentrations of albumin, bicarbonate, phosphate, and citrate. This correlation, however, falls apart in acute care clinical situations where patients are unable to maintain the normal amounts of these metabolites in their blood.\(^{12}\)

**Clinical Biochemistry of Ca\(^{2+}\)**

Total calcium in blood plasma is maintained within a relatively narrow range of 8.8 to 10.6 mg per dl (2.20 to 2.65 mmol per L). Ionized calcium (Ca\(^{2+}\)) levels are normally 4.40 to 5.40 mg per dl (1.10 to 1.35 mmol per L) or approximately 50 percent of the total calcium.\(^{16}\) Again, the relative distribution of ionized calcium, complexed calcium, and protein-bound calcium is altered by many factors. One of these important factors is pH. Acidosis, or a decrease in serum pH, causes an increase in the ionized calcium fraction, while alkalosis, an increase in serum pH, causes a corresponding decrease in ionized calcium. Because of this relationship, and the need to maintain the original pH of the specimen, it is important that an anaerobic specimen be obtained in a similar fashion as for blood gas analysis (heparinized arterial or venous whole blood samples are acceptable). The anaerobic sample can be stored or centrifuged at 4°C for up to two hours before changes in Ca\(^{2+}\) concentration are seen. Although the ionized calcium results may be mathematically corrected for changes in pH owing to the exposure of the specimen to air, the corrected or “pH normalized” (to pH 7.4) calculated ionized calcium result represents the ionized calcium value adjusted for the specimen pH change and is only an approximation of the true anaerobic ionized calcium value.\(^{17}\) Various nomograms used for calculating Ca\(^{2+}\) have been shown to be less than reliable, and none has seen widespread use in the clinical laboratory. Instead, physicians simply rely on Ca\(_T\), or better still Ca\(^{2+}\), as an indicator of calcium status in their patients.\(^{17}\) A decrease in plasma proteins can also result in a decreased total calcium value, while the ionized calcium level usually remains normal. Therefore, the measured ionized calcium value can more precisely characterize the physiologically active calcium state in patients with altered protein levels.

In blood, calcium is present almost exclusively in the plasma phase, and the concentration of plasma Ca\(^{2+}\) is maintained within narrow limits through hormonal control. The two most important hormones affecting calcium homeostasis are the vitamin D metabolite, 1,25-dihydroxycholecalciferol [1,25-(OH)\(_2\)D\(_3\)] and parathyroid hormone (PTH). Calcium homeostasis is determined by the amount of calcium ingested in the diet, the amount of dietary calcium absorbed into the bloodstream by the gut (primarily under the regulation of 1,25-(OH)\(_2\)D\(_3\)), and the amount of bone resorption, renal reabsorption, and urinary excretion of calcium (primarily under the regulation of PTH). Calcitonin, gonadal steroids, glucocorticoids, thyroid hormones, osteoclastic activating factor, growth factors, and prostaglandins are also involved in regulating plasma calcium levels, although to a much smaller degree than 1,25-(OH)\(_2\)D\(_3\) or PTH. In certain pathological conditions, other factors (e.g., interleukin-1, lymphotoxin, tumor necrosis factor) may cause significant disturbances in circulating levels of Ca\(^{2+}\).

\(^{17}\)
Sample Collection and Transport

Both whole blood, plasma, and serum are ideal for measuring ionized calcium. In either case, samples must be maintained anaerobically to ensure that the pH of the sample remains stable. The in vitro loss of carbon dioxide from the sample will cause an increase in pH and concomitant decrease in measured Ca$^{2+}$. Therefore, air bubbles should be avoided when heparinized syringes are used for collection of whole blood samples. Venous stasis has only a minimal effect on ionized calcium, but a few minutes of forearm exercise, with stasis, has a deleterious effect owing to the localized production of lactic acid (a calcium ligand). Thus, the patient should be in a relaxed state when blood is drawn. Whole blood ionized calcium is recommended over serum since use of serum requires time for clotting and centrifugation. Whole blood also has advantages in instances where the need for rapid results is indicated, e.g., monitoring Ca$^{2+}$ during cardiopulmonary bypass surgery or liver transplantation. In addition, whole blood samples may be necessary in screening and monitoring Ca$^{2+}$ in neonates where the amount of blood removed should be minimized. Samples collected with the anticoagulants citrate, ethylenediamine tetraacetic acid (EDTA), oxalate or other high-affinity chelators of calcium will result in erroneously low ionized calcium concentrations and must be avoided. Sodium or lithium heparinate are the ideal anticoagulants for collecting whole blood for Ca$^{2+}$. While the polyanion heparin binds some calcium, if the heparin content is kept constant at about 15 IU per ml of blood or lower, the effect on ionized calcium is usually less than three percent. Heparin usually has less effect on ionized calcium in whole blood than in plasma. Studies of paired samples in our laboratories compared ionized calcium values in three ml syringes containing dry lithium heparin at 10 IU per ml of blood versus liquid sodium heparin at about 40 International units per ml. Use of the liquid sodium heparin gave a consistent decrease in ionized calcium of about 0.4 mg per dl (table I).

Another major drawback in using liquid heparin as an anticoagulant is its effect on sample dilution. Again, the use of lyophilized (dry) heparin in the dead-space of the syringe will obviate any sample dilution effects. These pre-analytical effects can be minimized as the use of calcium-titrated heparin or lyophilized pre-heparinized syringes containing about 10 IU heparin per ml blood become routinely available.

Reference Intervals

The reference interval for Ca$^{2+}$ concentrations in whole blood (4.40 to 5.40 mg per dl [1.1 to 1.35 mmol per L]) is very narrow, having a span of only about 10 percent of its median concentration. Concentrations of Ca$^{2+}$ decline shortly after birth but increase by approximately 0.4 mg per dl (0.1 mmol per L) one week later. Children and adolescents have slightly higher ionized calcium values than adults ($p < 0.05$). In table II are listed mean whole blood Ca$^{2+}$ concentrations for different age groups.

<table>
<thead>
<tr>
<th>Liquid Heparin Conc.</th>
<th>Ca$^{2+}$</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.U./ml Blood</td>
<td>mg/dl</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4.68</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>4.32</td>
<td>7.7</td>
</tr>
<tr>
<td>100</td>
<td>3.80</td>
<td>18.8</td>
</tr>
<tr>
<td>300</td>
<td>2.72</td>
<td>41.9</td>
</tr>
</tbody>
</table>

* Data are presented showing the effects of increasing amounts of heparin on Ca$^{2+}$ in whole blood drawn from one healthy volunteer. Data on Ca$^{2+}$ are the mean of duplicate analysis.
TABLE II
Ionized Calcium in Blood of Children and Adults

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Mean, Ca²⁺ (mg/dl)</th>
<th>(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord blood</td>
<td>14</td>
<td>5.20 ± 0.24</td>
<td>(1.30 ± 0.061)</td>
</tr>
<tr>
<td>1 day</td>
<td>10</td>
<td>4.40 ± 0.24</td>
<td>(1.10 ± 0.059)</td>
</tr>
<tr>
<td>3 days</td>
<td>8</td>
<td>4.52 ± 0.20</td>
<td>(1.13 ± 0.051)</td>
</tr>
<tr>
<td>5 days</td>
<td>7</td>
<td>4.86 ± 0.21</td>
<td>(1.22 ± 0.053)</td>
</tr>
<tr>
<td>1 – 20 years</td>
<td>19</td>
<td>4.70 ± 0.28</td>
<td>(1.18 ± 0.069)</td>
</tr>
<tr>
<td>Adults</td>
<td>24</td>
<td>4.76 ± 0.25</td>
<td>(1.19 ± 0.062)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± s.d.

Pathological and Iatrogenic Changes in Ionized Calcium

In most clinical conditions which affect calcium metabolism, the plasma protein concentration and acid/base status of the patient are close to normal. Thus, there is a broad correlation between ionized and total calcium concentration over the range of plasma calcium from extreme hypocalcemia to overt hypercalcemia. There are, however, several clinical situations where the relationship deviates from normal. The first of these arises during massive transfusions when citrated blood is administered to the patient. Citrate and phosphate ions bind calcium and a lowering of ionized calcium levels may be anticipated. The overall effect is dependent, however, on the relative amounts of blood transfused, the amount of calcium salts added, and the type of calcium salt.9

Thus, either hypocalcemia or hypercalcemia may be induced, depending on the net composition and amount of blood transfused. The degree of hypocalcemia is dependent on the rate of citrated blood infused, how quickly peripheral stores of calcium are mobilized in response to the lowering of ionic calcium, and the rate of citrate clearance by the liver and kidneys.15 Patients who have underlying hepatic and renal diseases have higher baseline levels of lactate and citrate and are at greater risk for developing hypocalcemia. In general, patients who are given blood at rates that are greater than 90 ml per hr (citrate concentration of 14.6 mmol per L) can begin rapidly to develop clinical signs of hypocalcemia.15

In patients undergoing liver transplantation, the inability of the liver to metabolize citrate produces increased risks for hypocalcemia during the surgical and post-surgical period. During the anhepatic phase of the procedure, plasma ionized calcium levels can fall to as low as 40 percent of normal in spite of calcium supplementation. Plasma citrate levels concomitantly can rise from 20 to 100 times the preoperative levels, depending on the rate blood is transfused.18

Another cause of disturbances in the relationship between ionized and total calcium is renal failure where there is often a metabolic acidosis and an abnormal plasma biochemistry with respect to many ions. In such cases "corrected" total calcium measurements appear to be of very little value. Untreated patients with renal failure frequently, have ionized calcium values below normal.4 Hemodialysis can bring about profound changes in the concentration of ionized calcium depending on the calcium content of the dialysis fluid. This is of great importance in the management of renal bone disease where it is important to maintain a positive calcium balance without producing hypercalcemia and subsequent metastatic calcification.

Neonatal hypocalcemia7 has been reported to occur in 40 percent of infants at risk including (1) preterm infants, (2) infants with birth asphyxia, and (3) infants of insulin dependent mothers. Neonatal hypocalcemia is a potentially life-threatening situation if it is severe or prolonged, or if the patient has additional medical problems as hyaline membrane
disease, seizures, hypotension, anoxia, or hypoglycemia. Complications of neonatal hypocalcemia may result from administration of bicarbonate to correct the patient’s acidosis. Bicarbonate administration (acts as a calcium ligand) results in a rapid decrease in ionized calcium, but not in total calcium. Ionized calcium levels of less than 2.4 mg per dl (0.6 mmol per L) in the presence of hypomagnesemia have been associated with symptoms of hypocalcemia. During exchange transfusions with citrated blood supplemented with calcium, mean ionized calcium decreased from 4.0 mg per dl to 2.8 mg per dl, while total calcium increased from 8.8 mg per dl to 10.2 mg per dl. Four of eight infants with ionized calcium less than 2.8 mg per dl also had symptoms of tetany.

There is some controversy concerning the importance of increased levels of plasma bilirubin on the concentration of ionized calcium. One report suggests that deeply jaundiced patients do not have increased complexed fractions of calcium and that bilirubin is, therefore, unlikely to bind much calcium. A more recent study suggests that high levels of bilirubin added to umbilical cord serum in vitro significantly reduced the concentration of ionized calcium. The authors suggest that elevated serum bilirubin levels in newborn infants may expose them to an added risk of hypocalcemia. Clearly, further work is needed to elucidate this finding.

Hypocalcemia is a common occurrence in critically ill patients, especially those with sepsis and/or renal, cardiac, or pulmonary failure following surgery or in burn patients. Frequently, serum protein and albumin levels are lowered, acid-base abnormalities are present, and citrated blood products are administered. All of these effects make the interpretation of total calcium very difficult. Severe hypocalcemia also has been associated with diminished hemodynamic function in acutely ill patients. A significant improvement of cardiac perfusion in these subjects requires infusion of calcium ions (up to 90 ml per hr). Close monitoring of Ca$^{2+}$ is essential to prevent any deleterious effects of such a rapid calcium infusion.

The diagnosis of hyperparathyroidism is rarely based solely on serum calcium measurements alone, although, it is generally a good indicator. In most cases the observed hypercalcemia applies to both total and ionized calcium. At one time the term “normocalcemic hyperparathyroidism” was used to describe a small subgroup of patients with total calcium levels within the normal range. Subsequently, such patients have been shown to have elevated ionized calcium levels, perhaps as a result of their mild acidosis. In a large series of hyperparathyroid patients, Ladenson et al found that 11.3 percent had an elevated ionized calcium, but a normal total calcium. The so-called idiopathic calcium renal stone-former is often suspected as having a similar serum profile as that of the normocalcemic hyperparathyroid patient; however, this study revealed only one out of 181 stone-formers to have an elevated ionized calcium and a normal total calcium. Other situations where the relationship between ionized and total calcium is disturbed include cirrhosis and treatment with thiazide diuretics. Again the reasons for these changes are not known.

Table III has been modified from the original material found in an article by Bowers et al to reflect the current status of Ca$^{2+}$ measurements. These data and the studies that support them clearly indicate the diagnostic usefulness of ionized calcium.

Summary

Hypocalcemia is an important metabolic problem since if untreated, it may
TABLE III

Clinical Applications of Ionized Calcium (Ca\(^{2+}\)) *

A. Ca\(^{2+}\) can identify hypocalcemia that is missed by total calcium (Ca\(^{2+}\)) measurements alone

Examples:
1. Massive transfusion of citrated whole blood in patients with underlying hepatic or renal diseases
2. Infusion of fresh frozen plasma containing full concentrations of citrate
3. Endstage renal disease
   a. Significant pH and albumin changes can alter various calcium fractions, unpredictably
   b. Profound albumin loss can occur in nephrosis
   c. Monitoring and regulating dialysis baths and dialysis patients can obviate significant calcium shifts

B. Ca\(^{2+}\) can enhance diagnostic sensitivity and specificity of testing.

Examples:
1. Neonatal hypocalcemia is associated with:
   a. A relative hypoparathyroidism at birth
   b. The gestational age of premature infants
   c. Very ill newborns with tetany and/or seizures who require monitoring and therapy
   d. Acute changes after birth which may not require treatment
2. Malignancy
   a. Supports suspicion of neoplasm or granuloma, especially if parathyroid hormone is normal
   b. Monitors tumor-associated symptoms of hypercalcemia
2. Hyperparathyroidism
   a. Confirms diagnosis of normocalcemic hyperparathyroidism
   b. Monitors more sensitively the effectiveness of treatment


lead to serious cardiac, pulmonary, neurological, and metabolic sequelae. It is not an uncommon finding in critically ill patients and neonates. There is now a consensus that Ca\(^{2+}\) measurements are analytically accurate, and, in some patients, may be more physiologically and clinically meaningful than total calcium measurements. However, several factors affect the Ca\(^{2+}\) concentration and must be carefully controlled for the results to be meaningful. The most important of these considerations are to maintain the sample anaerobically, the need to measure pH concurrently and to control carefully the concentration of heparin in the whole blood sample.

References