State-of-the-Art Review

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Review of hypocalcemia in septic patients

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Abstract

Objective: To review the occurrence and etiologies of hypocalcemia in septic human and veterinary patients.
Data sources: A thorough search was conducted using CAB abstracts and MEDLINE and the keywords hypocalcemia, ionized calcium, sepsis, and procalcitonin (proCT).
Human data synthesis: Ionized hypocalcemia (iHCa) is a common finding in septic human patients. The cause is unknown but is likely multifactorial. Low ionized calcium (iCa^{2+}) concentrations coincide with increased severity of illness and increased mortality. Recent studies show that iHCa has a strong correlation with elevated calcitonin precursor concentrations.
Veterinary data synthesis: There is a paucity of publications in the veterinary literature pertaining to iHCa in septic animals. Experimental models of sepsis indicate that iHCa exists in animals. iHCa has also been investigated in horses with enterocolitis and endotoxemia. Prospective studies are needed to determine the prevalence of iHCa among septic small animals, and to determine whether iHCa correlates with increased mortality and severity of disease. Indications for the treatment of iHCa in septic small animals also need to be investigated.
Conclusions: iHCa is well documented in septic human patients, but little is known about iHCa in septic veterinary patients. Future veterinary studies should focus on documenting the presence of iHCa in septic patients and steps should be taken to determine the cause. proCT concentrations may show promise for predicting sepsis and mortality in critically ill veterinary patients.

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Introduction

Hypocalcemia is a widely recognized electrolyte disturbance in septic human patients. Since the late 1970s, hypocalcemia has been extensively researched in septic and other critically ill patients to determine its etiology and impact on case outcome.[1–14] Hypocalcemia has been documented in many types of critically ill patients including pediatric patients,[15] adult surgical patients,[9] and in patients with burn injuries,[16] pancreatitis,[17] and severe trauma.[13] Astoundingly, ionized hypocalcemia (iHCa) has been documented in as many as 88% of one intensive care patient population.[11] A strong correlation between sepsis and hypocalcemia is apparent in humans with incidence rates ranging from 20% to 50%.[3,4,10]

Hypocalcemia in septic animals is less well studied. Recently, hypocalcemia was documented in endotoxemic horses,[18] and in experimental studies of sepsis in rats,[19,20] pigs,[21,22] and hamsters.[23,24] These studies focus primarily on documenting the occurrence of hypocalcemia in septic animals and attempt to determine its etiology and repercussions. Hypocalcemia has been evaluated in various diseases in small animals; however, none include sepsis. Only 1 case report of iHCa in a septic dog exists in the literature.[25] Therefore, little is known about the incidence and repercussions of hypocalcemia in the critically ill small animal population.

At present, the cause of hypocalcemia in most critically ill and septic human and veterinary patients is unknown, but is suspected to be multifactorial. Suggested mechanisms include intracellular redistribution of calcium or accumulation of calcium within tissues, as well as hypomagnesemia and alkalosis.[3,11] In addition, abnormalities in calcium regulation such as parathyroid gland insufficiency or suppression, vitamin D deficiency or impaired response to vitamin D, elevations in

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calcitonin precursors, and cytokine-mediated down-regulation of parathyroid hormone (PTH) and PTH receptors have been postulated.[3,11] Treatment of hypocalcemia in both septic human and veterinary patients remains a controversial issue and recommendations vary from case to case.

Calcium is essential for a multitude of processes that occur throughout the body. Normal cardiovascular function, intestinal motility, neuronal conduction, mitotic division, and muscle contraction all rely on adequate calcium concentrations.[26] Enzyme activity, hormone secretion, blood clotting, and bone metabolism also depend on normal calcium stores.[26,27] Without adequate calcium regulation, the body ceases to function properly and clinical consequences occur including decreased wound healing, cardiac contractility, and systemic vascular resistance.[26,27] In experimental and clinical studies in both humans and animals, hypocalcemia is associated with a greater severity of illness and correlates with increased mortality rates.[2,3,6,7,9–11,20,24] Therefore, recognition of this electrolyte abnormality and its etiologies may have implications for the future diagnosis and treatment of sepsis in animals.

Regulation of Calcium

Less than 1% of calcium in the body is considered readily available, whereas the remainder is stored along with phosphate in bone.[28] In normal dogs, approximately 56% of total serum calcium is in the ionized form.[29] The ionized form is the most clinically important as it is physiologically active and tightly regulated. Thirty-four percent of calcium is protein bound, the majority of which is bound to albumin.[29] The remaining 10% of total serum calcium is chelated with citrate, phosphate, bicarbonate, lactate, and free fatty acids.[27] The percentage of calcium in each form changes depending on therapies and drugs administered, or the disease process that is present, such as protein-losing diseases or alterations in acid–base status.[27] Transfusions with blood that contain citrate as an anticoagulant will cause a transient decrease in ionized calcium (iCa^{2+}) concentrations as the citrate chelates the calcium. Similarly, both lactic acidosis and metabolic alkalosis will cause iHCa as both lactate and bicarbonate also chelate calcium. Also, alkalosis leads to an increase in anion groups available for binding, which causes an increase in the protein-bound fraction of calcium and a subsequent decrease in iCa^{2+}.

Circulating levels of iCa^{2+} are kept in a narrow range by the actions of PTH, calcitriol (1,25-dihydroxyvitamin D_3), and calcitonin. PTH and calcitriol are the major regulators of calcium homeostasis and increase calcium concentrations by mobilizing calcium from bone, increasing gastrointestinal absorption of calcium, and decreasing renal excretion of calcium.[28] PTH is responsible for the minute-to-minute control of serum iCa^{2+}, while calcitriol upholds the day-to-day control.[28] Calcitonin decreases calcium concentrations by decreasing calcium mobilization from bone and functions as an emergency regulator for states of life-threatening hypercalcemia.[28] Within seconds of detecting mild iHCa, a marked increase in PTH secretion occurs. This stimulates renal calcium reabsorption, phosphorus excretion, and bone mobilization of calcium and phosphate. After several hours of iHCa, increased PTH secretion stimulates the synthesis and release of calcitriol. This causes increased absorption of calcium and phosphorus from the intestines. Following days or weeks of iHCa, hypertrophy and hyperplasia of the parathyroid gland lead to further increases in PTH secretion.[28]

Causes of Hypocalcemia in Sepsis

iHCa is associated with many diseases in small animals (Table 1). The mechanisms for iHCa in these patients are fairly well understood and are beyond the scope of this article. Conversely, the cause of iHCa in septic patients remains largely unknown and is likely multifactorial (Table 2). Suggested mechanisms include alterations in the release and action of PTH, increased urinary loss of calcium, calcium chelation, tissue or cellular calcium accumulation, hypomagnesemia, alkalosis, and, most recently, elevations in calcitonin precursors.

Ideally, PTH concentrations should be measured in patients with iHCa to evaluate whether the body is responding appropriately to the low iCa^{2+}. Impaired PTH synthesis occurs due to primary or secondary hypoparathyroidism. Primary hypoparathyroidism happens occasionally in dogs and rarely in cats;[28] however, secondary hypoparathyroidism is more common and has been documented in humans with sepsis.[3] It has been suggested that secondary hypoparathyroidism is part of a multiple organ failure syndrome that results in dysfunction of the parathyroid glands.[27] Another proposed mechanism for secondary hypoparathyroidism is suppression of the parathyroid glands via direct or indirect effects of circulating inflammatory mediators such as cytokines.[30] Elevated cytokine concentrations including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1 correlate inversely with iCa^{2+} concentrations in humans[6] and horses[31,32] with sepsis. In horses and cows, IL-1 and IL-6 have been found to decrease PTH secretion.[33,34]

The term 'relative hypoparathyroidism' may be applied to patients in whom PTH concentrations are normal in the face of concurrent iHCa; that is, the PTH

Table 1: Conditions associated with ionized hypocalcemia in dogs and cats

Diseases	Treatments
Diabetes mellitus	Phosphate enema administration
Acute and chronic renal failure	Sodium bicarbonate administration
Eclampsia	Improper sample anticoagulant
Acute pancreatitis	Phosphate administration
Severe trauma or rhabdomyolysis	Multiple blood transfusions
Hypoparathyroidism	Excessive intravenous fluid administration
Ethylene glycol toxicity	
Intestinal malabsorption	Furosemide administration
Hypovitaminosis D	
Hypomagnesemia	
Nutritional secondary hyperparathyroidism	
Tumor lysis syndrome	
Feline lower urinary tract obstruction	
Sepsis	

Table 2: Suggested mechanisms for ionized hypocalcemia during sepsis

A. Secondary hypoparathyroidism
  a. Multiple organ dysfunction syndrome causing parathyroid gland dysfunction
  b. Cytokine mediated suppression of the parathyroid glands
B. Hypomagnesemia
  a. Impaired PTH secretion
  b. End-organ PTH resistance
  c. Impaired renal vitamin D activation
C. Vitamin D deficiency or lack of activation of vitamin D
D. Decreased bone turnover*
E. Increased renal calcium excretion*
F. Chelation of calcium (lactate, bicarbonate)
G. Metabolic and respiratory alkalosis
H. Accumulation of calcium in tissues or cells
I. Elevation in calcitonin precursors (procalcitonin)

*No longer thought to have an important role.
PTH, parathyroid hormone.

concentrations are not as high as would be expected in a hypocalcemic patient. PTH concentrations seem to vary among septic patients and between different studies. In a study evaluating horses with enterocolitis and presumed endotoxemia, 70.6% of horses with ionized hypocalcemia demonstrated inappropriately low elevations in PTH.[35] This was believed to be the result of impaired parathyroid gland function. Conversely, Toribio et al.[36] demonstrated variable PTH measurements in mares injected with *Escherichia coli* lipopolysaccharide (LPS) as horses demonstrated both increases and decreases in PTH concentrations.

Elevated PTH concentrations have been documented in humans with sepsis and iHCa; however, the fact that the elevated PTH concentration does not restore the iCa^{2+} concentration to normal suggests that there is some other counter-regulatory mechanism present that prevents normalization of iCa^{2+} concentrations.[6] Interestingly, one study found that in critically ill surgical patients, nonsurvivors tended to have low iCa^{2+} concentrations with PTH concentrations more than double that of surviving patients.[9] Conversely, surviving patients tended to have PTH concentrations closer to the normal range. Therefore, low iCa^{2+} concentrations with elevated PTH concentrations were early predictors for death in critically ill surgical patients. It is unknown whether or not the elevated PTH concentrations were merely an indication of the severity of the iHCa or if there was another unknown etiology that was elevating the PTH concentrations. PTH secretion is also stimulated by β-adrenergic stimulation of the chief cells in the parathyroid gland by catecholamines, which are elevated in critically ill animals, including those with sepsis.[37]

Hypomagnesemia is also a suspected cause of iHCa in septic humans. Low serum magnesium concentrations contribute to iHCa via several mechanisms including impaired PTH secretion, end-organ PTH resistance, and decreased activated vitamin D. Magnesium deficiency in humans is associated with low serum PTH concentrations and supplementation with magnesium increases PTH concentrations to normal.[38–40] This is due to magnesium's vital role in calcium homeostasis. PTH is normally released from the parathyroid gland following activation of the adenylate cyclase enzyme that enables production of the second messenger cyclic adenosine 3′5′-monophosphate (cAMP).[38,39,41] Magnesium modulates adenylate cyclase activity, thereby facilitating the production of cAMP, which then stimulates the release of PTH.[41] Hypomagnesemia exacerbates the inhibitory effect of calcium on adenylate cyclase, causing decreased cAMP generation and therefore decreased PTH release.[41] Also, hypomagnesemic humans seem to have a decreased response to PTH in the skeletal system and kidneys due to reduced cAMP production and decreased activity of phosphoinositide-specific phospholipase C.[38,39] Lastly, because magnesium is required for hydroxylation (activation) of vitamin D in the kidneys, as well as PTH release, which also stimulates vitamin D activation, magnesium deficiency can reduce serum 1,25-dihydroxyvitamin D concentrations thereby leading to decreased intestinal calcium absorption.[41,42] It is important to remember that serum magnesium represents $<1\%$ of total body magnesium, thereby making accurate assessments of magnesium status difficult.[43] Ionized magnesium represents 63% of total serum magnesium in the dog and is the physiologically active form.[43] Therefore, ionized magnesium

concentration may be a more accurate reflection of magnesium status in critically ill or septic patients.

iHCa may also result from the lack of calcitriol activation or from calcitriol deficiency.[3] This occurs occasionally in critically ill patients due to malnutrition or malabsorption. Animals with sepsis may have a dietary vitamin D deficiency or the inability to hydroxylate vitamin D in the liver or kidneys. Renal hydroxylase deficiency has been described as a cause of iHCa in human patients with sepsis.[3]

Although previously hypothesized, recent studies show that iHCa in septic patients is not the result of attenuated bone resorption of calcium or increased urinary calcium excretion.[6,10] Studies in both septic humans and horses have failed to show increases in urinary calcium concentrations during episodes of iHCa, thus disproving the theory that renal losses contribute to the hypocalcemic state.[6,10,36]

Calcium chelation with lactate or bicarbonate has also been proposed to occur in septic patients with iHCa.[30] iHCa has been documented in human patients that have lactic acidosis secondary to poor perfusion from hypovolemia during septic shock.[44] iHCa also develops in patients with alkalosis due to elevations in the amount of calcium bound to protein. In dogs with experimentally induced alkalosis, both acute metabolic and respiratory alkalosis induced iHCa.[45] The iHCa was more marked in metabolic alkalosis compared with respiratory alkalosis, and correlated with decreased PTH secretion. Even when the iHCa was corrected to normal with calcium supplementation, the PTH concentrations remained decreased, suggesting a direct suppressive effect of the alkalosis on PTH secretion. However, the possibility that the administration of calcium exerted a suppressive effect on PTH release cannot be ruled out as part of the cause for the low PTH concentrations. It is speculated that decreased PTH secretion facilitates bone calcium deposition, thus reducing iCa^{2+} concentrations. Similarly, because septic patients have an increase in sympathetic tone and catecholamine release, they exhibit an increase in the release of free fatty acids from adipose tissue.[46] Theoretically, free fatty acids may also chelate calcium resulting in iHCa.

Intracellular, tissue, and third space accumulation of calcium have all been implicated as possible mechanisms for iHCa in septic patients. Increases in intracellular calcium occur due to the influx of iCa^{2+} from the extracellular compartment, as well as decreased cellular efflux of iCa^{2+}, which have both been documented to occur during sepsis and potentiate cellular death.[47] Suggested causes for these include IL-1-mediated flux across cell membranes[48] and impaired calcium-dependent ATPase activity.[49] However, many believe that because the intracellular calcium content is so small in comparison with the extracellular calcium content, the amount of calcium required to enter the cell and cause damage is minimal and would not be enough to cause major changes in extracellular iCa^{2+} concentrations.[10] The accumulation of calcium in the interstitium or other body spaces seems more plausible since the interstitial and third space fluid volumes are much greater than the blood volume. In septic humans, elevated calcium concentrations have been found in hepatocytes and aortic smooth muscle.[50] In septic rats, calcium accumulated in hepatocytes,[51] and in endotoxemic pigs, both abdominal fluid and liver had elevated calcium concentrations.[22] Conversely, other studies in septic patients have demonstrated a decrease in calcium concentrations in a variety of tissues.[50] Overall, it appears that intracellular or interstitial calcium accumulation may be a feasible hypothesis and should be considered when evaluating the mechanisms by which iCa^{2+} concentrations decrease in septic patients.

Recently, studies in human medicine have focused on the presence of elevated calcitonin precursors (procalcitonin [proCT]) in septic patients.[5,7,12–14] Decreased iCa^{2+} concentrations in septic human patients correlate with increased proCT concentrations, especially in patients with positive blood cultures.[5,7,12–14] Elevations in proCT concentrations become more pronounced as the severity of infection increases.[5,7,12] However, mature calcitonin concentrations, which would normally facilitate the lowering of iCa^{2+} concentrations, remain within the normal range during sepsis in these patients. The source and function of proCT in sepsis is poorly understood. Usually, production of proCT occurs only in the C cells of the thyroid; however, in experimentally induced sepsis in hamsters, proCT concentrations are elevated several fold in the plasma and in nearly all tissues in the body.[52] Also, calcitonin mRNA is ubiquitously and uniformly expressed in multiple tissues during sepsis. Thus, it appears that sepsis creates a host response that upregulates the production of proCT. A study in humans also evaluated the expression of calcitonin mRNA in adipose tissue from normal noninfected patients and patients with sepsis.[53] Adipose tissue from patients with sepsis demonstrated calcitonin mRNA; the first time that calcitonin mRNA has been documented in humans outside of the thyroid gland. Interestingly, proCT does not appear to play a role in calcium regulation in normal individuals, in comparison with its mature counterpart that facilitates lowering iCa^{2+} concentrations.[52] Thus, while it would seem logical that elevations in proCT are a direct cause of the iHCa in septic patients, this is not likely the case. Calcitonin precursors may play a role in the development of iHCa; however, the pathophysiologic mechanism remains to be determined.

Current Human Research

Several human studies document the incidence of hypocalcemia in septic patients and have also shown that the presence of hypocalcemia portends a worse outcome. Unfortunately, because sepsis has been defined differently among investigators in the past, it is difficult to draw comparisons between studies. However, the information in each individual study is intriguing and when evaluating the studies as a group, they show trends that support the importance of iCa^{2+} in the critically ill population. Desai et al.[10] evaluated the calcium status of 88 patients upon presentation to a medical intensive care unit (ICU). Seventy percent of all patients had iHCa and 81.6% (40/49) of patients with sepsis had iHCa. The mortality of patients with iHCa (44%) was significantly greater than the mortality of normocalcemic patients (17%). In another study, the records of 80 confirmed bacteremic patients and 80 patients with fever and suspicion of infection that were nonbacteremic were evaluated retrospectively to determine that 37.3% of bacteremic patients had low serum total calcium concentrations compared with 4.6% of nonbacteremic patients.[4] In the bacteremic patients, the incidence and magnitude of the serum total hypocalcemia in gram-negative and gram-positive infections were similar, and hypocalcemic patients had a significantly higher maximal body temperature. Conversely, Zaloga and Chernow evaluated serum iCa^{2+} concentrations in 60 patients with bacterial sepsis at the time of admission to an ICU.[3] Twelve of 60 (20%) critically ill patients with bacterial sepsis had iHCa. In this study, only patients with gram-negative sepsis had iHCa. The mortality rate was significantly higher in septic patients with iHCa (50%) compared with normocalcemic patients with sepsis (29%). Other studies have also confirmed that iHCa is significantly correlated with sepsis in human critically ill patients.[6–8]

iCa^{2+} has also been considered a useful tool to predict mortality in the general critically ill human patient population. Chernow et al. evaluated 210 patients admitted to the ICU, 64% of whom had iHCa. Patients with iHCa spent a significantly longer time in the ICU and had a significantly increased mortality rate (13.0%) compared with those who were normocalcemic (1.3%).[2] Recently, early iHCa was evaluated in 212 consecutive patients with severe trauma and revealed that patients with the most profound decreases in iCa^{2+} concentrations had sustained more severe trauma and more frequently suffered prehospital cardiac arrest.[54]

Recent studies also show that iHCa is related to increased circulating levels of inflammatory cytokines and increased severity of disease. Lind et al.[6] evaluated 26 patients admitted to the ICU, half of whom suffered from sepsis and half who had undergone major surgery. The septic patients exhibited a more pronounced iHCa that lasted for a longer period of time compared with the nonseptic surgical patients. TNF-α, IL-6, and C-reactive protein were markedly elevated in the septic patients and were inversely correlated with iCa^{2+} concentrations. Carlstedt et al.[7] also evaluated calcium status in septic and surgical patients and found that iCa^{2+} concentrations were significantly related to the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, confirming that iHCa was more pronounced with increasing severity of disease. Interestingly, PTH concentrations were also significantly related to the APACHE II scores of patients in this study and patients with elevated PTH levels had a significantly poorer outcome.

The most recent topic of interest relating to calcium status in critically ill and septic human patients is the relationship between iHCa and elevations in proCT concentrations. Many current studies show that human septic patients with decreased iCa^{2+} concentrations have increased proCT concentrations.[5,7,12–14] ProCT concentrations are elevated especially in patients with positive blood cultures and elevations in proCT increase as the severity of infection worsens.[5,7,12] Assicot et al.[12] evaluated serum proCT concentrations in 79 children hospitalized for suspected infections. Patients with severe bacterial infections had very high serum proCT concentrations at diagnosis and serum proCT concentrations decreased rapidly with antibiotic therapy. Similarly, among 9 patients with severe burns, proCT concentrations were closely related to infectious complications and acute septic episodes.[12] Concentrations of mature calcitonin were also measured and were normal in all subjects regardless of the degree of elevation in proCT concentrations. Meisner et al.[55] evaluated 40 patients with sepsis or systemic inflammatory response syndrome (SIRS) and found that the sepsis-related organ failure assessment (SOFA) score was significantly associated with higher proCT concentrations when evaluated over 15 days of hospitalization. Similarly, Castelli et al.[56] demonstrated that in 150 adult intensive care patients proCT concentrations were significantly correlated with SOFA scores and were highest in patients with infection. Thus, measurement of proCT during sepsis may provide insight into the severity and course of the disease.

A retrospective study evaluating 405 trauma patients compared the severity of injury, development of infectious complications, and organ dysfunction in relation to proCT concentrations.[13] ProCT concentrations correlated with the severity of trauma and patients who developed SIRS demonstrated a significant increase in peak proCT concentrations compared with patients

without SIRS. Likewise, increased proCT concentrations during the first 3 days after trauma predicted the development of severe SIRS, sepsis, and multiple organ dysfunction syndrome. Whang et al.[5] also determined that serum proCT concentrations could be used as a screening test for early detection of sepsis in humans and that peak concentrations correlated with prognosis. Similarly, Muller et al.[14] found that proCT was the most reliable laboratory variable for the diagnosis of sepsis compared with C-reactive protein, IL-6, and lactate, and that elevations in proCT were associated with a poor prognosis. Recently, proCT has been used successfully as a prognostic marker of outcome in ventilator-associated pneumonia[57] and was previously shown to distinguish septic and nonseptic causes of acute respiratory distress syndrome.[58] Also, measuring proCT concentrations in patients with suspected lower respiratory tract infection was used to guide antibiotic use in a prospective randomized clinical trial.[59] The patients in the proCT-guided group had significantly reduced use of antibiotics; when compared with patients in the standard group that received the usual full course of antibiotics there was no difference in outcome.

Current Veterinary Research

Few studies are available addressing iHCa specifically in septic veterinary patients. Most studies involving animals are experimental models of sepsis evaluating the presence of iHCa and its clinical effects. In rats, experimentally induced sepsis resulted in iHCa, the degree of which correlated with mortality.[20] Similarly, calcitonin precursors have been evaluated in hamster models of sepsis and have demonstrated that elevated concentrations of proCT are an early systemic marker of sepsis that correlate with low serum total calcium concentrations and increased mortality.[24] Nylen et al.[23] also evaluated proCT concentrations in hamsters and demonstrated that increased proCT concentrations correlated with increased mortality in experimental sepsis; however, neutralization of proCT with antiserum resulted in reduced mortality rates. This suggests that proCT is not just a marker for sepsis and systemic inflammation, but may also be a mediator, thereby making it a useful indicator for predicting outcome, in addition to being a potential target for therapeutic intervention. Recently, an abstract described the use of a human proCT assay to detect proCT in dogs with SIRS and suspected sepsis.[a] Three healthy dogs served as controls and had negative proCT tests. Among 18 SIRS dogs, 15 (83%) had positive proCT tests. Blood cultures were used to confirm sepsis and proCT results were compared with blood culture findings. Results showed that proCT concentrations were elevated in dogs with negative blood cultures and vice versa. Therefore, proCT concentrations did not allow differentiation between septic and nonseptic SIRS dogs in this study.

There are no published reports of clinical studies evaluating the incidence of iHCa in septic small animals and how it may correlate with mortality. However, hypocalcemia has been associated with increased mortality rates in other disease states. Calcium status was evaluated in 80 cats with chronic renal failure and revealed that iCa^{2+} concentrations were significantly lower in end-stage chronic renal failure.[60] Suggested mechanisms for this include reduced glomerular filtration rate and reduced intestinal calcium absorption following a decrease in calcitriol concentration. In a retrospective study evaluating 46 cats diagnosed with acute pancreatitis, those with plasma iCa^{2+} concentrations less than or equal to 1.00 mmol/L had a poorer outcome than those with higher plasma iCa^{2+} concentrations.[61] Similarly, a retrospective study evaluated acute renal failure in 99 dogs and found that serum total calcium concentrations <8.6 mg/dL portended a poorer prognosis for survival and discharge from the hospital.[62] If hypocalcemia is associated with increased mortality and prolonged hospitalization in these disease states, it is possible that the same relationship may exist in septic veterinary patients.

A current literature search revealed just 1 case report of iHCa in a septic dog.[25] The report described a 12-year-old spayed female English Sheepdog that developed septic shock 5 days following hemilaminectomy surgery. *Streptococcus canis* was cultured from the incision site. Seven days following surgery, the dog developed muscle tremors and an iCa^{2+} level of 0.43 mmol/L (reference range 1.12–1.4 mmol/L) was measured. The dog was treated with a constant rate infusion of calcium gluconate and remained on the infusion for the duration of her hospital stay, because she became ionized hypocalcemic each time calcium supplementation was discontinued. PTH concentrations were measured 8 days following surgery and were normal at 10.4 pmol/L (reference range 2–13 pmol/L) while the iCa^{2+} remained low at 1.15 mmol/L (reference range 1.25–1.45 mmol/L). It could be argued that for the degree of iHCa, the PTH level should have been higher, suggesting a relative hypoparathyroidism. However, partial suppression of PTH may have occurred due to previous and concurrent calcium supplementation. No cause for the iHCa could be determined aside from the documented sepsis. Despite aggressive treatment, the dog was euthanized 2 weeks after surgery and a postmortem exam revealed fibrin emboli with bacterial colonies in the heart, lungs, and spleen.

In recent years, iHCa has been researched more extensively in horses. Toribio et al.[35] found that 75% of

horses with enterocolitis had iHCa and ionized hypomagnesemia. Another group of researchers discovered that 86% of horses had iHCa before undergoing colic surgery.[63] In horses with strangulating lesions of the gastrointestinal tract, the iCa^{2+} was significantly lower than in horses with nonstrangulating lesions.[63] The authors suggest that endotoxins, important mediators in severe enterocolitis in horses, play a role in the resulting iHCa. Recently, Toribio et al.[36] evaluated electrolyte changes in mares injected with LPS, and found significant decreases in both iCa^{2+} and ionized magnesium concentrations.

Clinical Manifestations of Hypocalcemia in Sepsis

Because calcium is required for a large number of physiologic processes, many different clinical signs can be seen during hypocalcemia (Table 3). Clinical signs tend to be the same, regardless of the underlying cause of the hypocalcemia. Because iCa^{2+} increases the threshold of excitability for neurons, low serum iCa^{2+} concentrations result in signs of excitation. The severity of clinical signs is determined by the magnitude of the hypocalcemia, as well as the duration and rapidity of onset.[28] Acute hypocalcemia usually results in severe clinical signs, whereas clinical signs may be less obvious if the development of hypocalcemia occurs slowly. Animals with mild hypocalcemia often display no clinical signs.

Several studies have attempted to determine the cardiovascular consequences of sepsis and hypocalcemia. Human and veterinary patients with sepsis often have decreases in systemic vascular resistance and myocardial function. The reduction in myocardial function is reversible and is characterized by a diminished left and right ventricular ejection fraction, ventricular dilation, and altered diastolic pressure–volume relationships.[64,65] Although sepsis-induced changes in myocardial function can be marked, a low cardiac index is very uncommon, even in the late stages of septic shock.[65] When iHCa is present concurrently with sepsis, the decreases in cardiac contractility may be more pronounced.[66] This is likely due to the important role that calcium plays in excitation–contraction coupling, which enables cardiac myocyte function. In addition, iHCa increases cardiac cell membrane excitability by decreasing the degree of depolarization necessary for an action potential to occur. Consequently, electrocardiographic changes that have been demonstrated with hypocalcemia include tachycardia and prolonged QT intervals.[28] Severe decreases in serum total calcium may lead to myocardial failure and death.[28]

Smooth muscle tone is determined by the calcium-dependent phosphorylation of the myosin light chain.

Table 3: Clinical manifestations of hypocalcemia in animals

	Cardiovascular
Muscle tremors/ fasciculations	
Muscle cramping	Tachycardia*
Stiff gait	ECG changes (prolonged QT interval)*
Facial rubbing	Hypotension*,†
Restlessness/excitation	Cardiopulmonary arrest
Panting	
Lethargy*	
Tetany	
Seizures	
Hyperthermia	

*Seen most commonly with concurrent sepsis.
†Only documented in human patients.

Thus, intracellular calcium is necessary to achieve smooth muscle contraction to maintain vasoconstriction. An association between iHCa and hypotension has also been found among human intensive care patients. Desai et al.[67] evaluated 112 ICU patients and found that hypotensive patients had significantly lower iCa^{2+} concentrations. Additionally, vasopressor support was required in 41% of hypocalcemic patients, compared with 14% of normocalcemic patients.[67] Therefore, iHCa should be considered as a differential cause of hypotension in septic patients that is refractory to fluid therapy and vasopressors. The effect of iHCa on blood pressure in veterinary patients is not known at this time.

Evaluation of Hypocalcemia

With the advent of portable clinical analyzers in many veterinary hospitals, iCa^{2+} has become a more commonly evaluated laboratory parameter. However, many clinicians still rely on serum total calcium to evaluate calcium status in their patients, and also use a correction formula to normalize serum total calcium concentrations that are suspected to be low due to concurrent hypoalbuminemia. The correction formula most commonly used is: serum total calcium − serum albumin + 3.5 = corrected serum total calcium.[68] Unfortunately, this formula was derived using a different analytic method from that used by many analyzers today, and the normal range for serum albumin used in this report was substantially lower.[28] Although a positive correlation exists between serum total calcium and serum albumin, 33% of the variability in dogs[68] and only 18% in cats[69] can be attributed to serum albumin. Therefore, the correlation between serum total calcium and albumin is weak, and varies considerably from patient to patient.[68–70] Consequently, the application of correction formulas has been found to be inaccurate

and obtaining iCa^{2+} concentrations to assess calcium status is highly recommended.

Recently, Schenck and Chew evaluated the correlation between serum iCa^{2+} concentrations and serum total calcium concentrations adjusted by correction formulas.[71] A total of 1633 canine serum samples were evaluated. Adjusted serum total calcium concentrations were inaccurate in predicting serum iCa^{2+} concentrations in 37% of all dogs evaluated. In dogs with chronic renal failure, the discordance between adjusted serum total calcium and iCa^{2+} concentrations increased to 55%. Serum total calcium concentrations consistently overestimated normocalcemia and underestimated hypocalcemia. Therefore, serum total calcium concentrations, even with the use of the adjustment formula, are unacceptable for predicting iCa^{2+} status in dogs.

Similar findings have been reported in human critically ill patients in which serum total calcium concentrations have a poor correlation with iCa^{2+} concentrations.[72–75] One study compared corrected serum total calcium concentrations with iCa^{2+} concentrations in 1040 critically ill surgical patients.[72] Over 38% of calcium concentrations were incorrectly determined by the adjustment formula. Similarly, an underestimation of hypocalcemia was noted when the correction formula was applied. Slomp et al.[73] had similar findings among 53 paired samples from patients requiring intensive care treatment. Adjusted calcium concentrations only rarely demonstrated hypocalcemia. Clearly, serum total calcium concentrations and adjusted total calcium concentrations are an inaccurate method of evaluating iCa^{2+} status in critically ill patients and are not recommended.

Improper sample handling will also lead to false results for iCa^{2+} measurements. It is widely known that EDTA will bind iCa^{2+}, leading to a false iHCa. However, the same is true when heparin is used improperly for sample anticoagulation. A recent study by Hopper et al.[76] found that heparin interferes with partial pressures of oxygen and carbon dioxide, base deficit, bicarbonate, potassium, sodium, chloride, lactate, and iCa^{2+} measurements. This is primarily the result of dilution from the use of excessively large volumes of heparin to effectively anticoagulate the blood sample. iCa^{2+} values are also lowered due to chelation of the divalent positive calcium ions with the strongly negative heparin molecules. Sodium heparin concentrations >15 IU/mL in blood samples form complexes with calcium ions and result in false decreases in measured iCa^{2+}.[77] Depending on the concentration, this may produce an error of up to 0.15 mmol/L of iCa^{2+}, which is significant considering the narrow reference range for normal iCa^{2+} concentrations.[78] Therefore, the ideal way in which to measure iCa^{2+} is to use syringes that contain only 2–3 IU/mL of heparin in a 'puff' of inert filler material that dissolves rapidly in the blood sample. This small amount of heparin is sufficient to provide anticoagulation, but is too small to complex with iCa^{2+} or dilute the sample.

Treatment

Treatment of hypocalcemia in septic human and veterinary patients remains controversial. Recommendations vary depending on the underlying disease process and status of the patient. Unfortunately, controlled clinical studies evaluating the use of calcium therapy in both human and veterinary septic patients have not been completed, leaving clinicians to question the efficacy and safety of calcium administration during sepsis. Kovacs et al.[66] demonstrated that calcium administration improved systolic and diastolic function in rats and that no adverse effects of calcium administration were seen, even at supraphysiologic concentrations. Similarly, Steinhorn et al.[79] revealed that calcium administration initially reversed decreases in cardiac output in experimentally induced sepsis in dogs. However, several other experimental studies have documented that calcium administration increases mortality in septic animals and does not improve hemodynamics or mortality rates.[20–22,80] Recently, Carlstedt et al.[22] showed that when calcium administration was used to restore iCa^{2+} concentrations to normal, neither hemodynamics nor survival was improved in the pigs with endotoxin-induced sepsis. Similarly, calcium administration increased mortality in endotoxin-treated rats[80] and in rats with septic peritonitis induced by cecal ligation.[20]

Because calcium is required to maintain myocardial function and vascular tone, it seems intuitive that supplementing calcium in cases of concurrent iHCa and septic shock would be beneficial. Unfortunately, septic patients may have an increased cellular influx of calcium that is deleterious to cellular viability and function.[79] Therefore, the administration of exogenous calcium may exacerbate the already elevated concentrations of intracellular calcium and promote cell death. Because many animals with sepsis respond adequately to fluid resuscitation and vasopressor therapy, it is recommended to pursue these forms of cardiovascular support before calcium supplementation.[21,79] Exceptions include patients demonstrating clinical signs consistent with hypocalcemia and patients with severely low iCa^{2+} concentrations.[28,81,82] In addition, some authors have recommended treating patients with concurrent hyperkalemia, documented vitamin D or PTH deficiency, or animals in which chelation has occurred such as those receiving blood transfusions or patients that are severely alkalotic.[28,79,81] Calcium supplementation should also be considered for patients

with documented iHCa whose hypotension is unresponsive to fluid resuscitation and vasopressor therapy. Rosol et al.[28] recommended administering 10% calcium gluconate at a dose of 0.5–1.5 mL/kg IV to effect or 5–15 mg/kg/hr IV if a continuous infusion is necessary. A continuous infusion is preferable to intermittent injections as an infusion will reduce peaks in calcium levels that may stimulate calcitonin release, thereby causing the calcium to decrease again.[79] A recent study evaluating calcium supplementation in human critically ill multiple-trauma patients found that individual response to calcium replacement therapy was highly variable, especially in patients with moderate to severe iHCa.[83] Therefore, frequent monitoring of iCa^{2+} concentration is recommended when attempting to normalize calcium concentrations in hypocalcemic patients.

Applications to Veterinary Emergency and Critical Care

Owing to the paucity of published literature describing hypocalcemia in septic veterinary patients, the incidence and consequences of iHCa in small animals is unknown. If septic veterinary patients are similar to humans, animals with sepsis are likely to be hypocalcemic. In addition, if hypocalcemia is present in septic small animal patients, those animals may have a poorer outcome. iCa^{2+}concentrations should be evaluated in all critically ill veterinary patients including those with sepsis, especially in animals displaying signs of hypocalcemia. Because little is known about the impact of calcium supplementation in septic patients, it is difficult to make recommendations for treatment of hypocalcemic patients with sepsis. However, because iHCa correlates with a poorer prognosis in many disease states including sepsis, normalization of iCa^{2+} concentrations may help to improve mortality rates. Calcium supplementation may be beneficial in septic patients with moderate to severe iHCa (<0.75 mmol/L) or in patients displaying clinical signs of hypocalcemia.

Recommendations for Future Studies

Currently, very little is known about iCa^{2+} in critically ill veterinary patients or in veterinary patients with sepsis. iCa^{2+}concentrations should be evaluated prospectively in the critically ill veterinary population to determine if certain patient groups are more likely to develop hypocalcemia. Then, septic patients and other critically ill patient groups tending towards iHCa should be investigated to determine whether iCa^{2+} concentrations correlate with increased mortality or prolonged hospitalization. Furthermore, studies should attempt to determine the etiology of the iHCa by evaluating serum PTH concentrations, urinary excretion of calcium, calcium concentrations in body fluids and tissues, and calcitonin and proCT concentrations. Special attention should be paid to acid-base status, magnesium concentrations, and renal function in these patients in an attempt to rule out concurrent causes of iHCa. Large-scale prospective randomized trials must also be performed to determine if calcium supplementation in hypocalcemic septic patients has any impact on patient outcome.

Conclusion

Hypocalcemia has become a widely recognized electrolyte abnormality in human critical care medicine, and has been researched extensively in many critically ill patients, including those with sepsis. Although hypocalcemia is not well documented in septic veterinary patients, it is likely that it may exist. The etiology of hypocalcemia in septic patients is not known; however, it is suspected to be multifactorial and likely varies among patients. Whether hypocalcemia is a protective mechanism that the body undergoes to reduce cellular damage during the inflammatory response, or is an indication of disease severity and thus a predictor of mortality, remains to be established. However, clinical repercussions of iHCa may occur; animals may become so severely affected that clinical signs of iHCa develop or recovery is compromised. Treatment continues to be a controversial issue and will likely remain so until further studies can be completed to evaluate hypocalcemia in septic veterinary patients. Hopefully, as the measurement of iCa^{2+} becomes more widely available and the awareness of this abnormality increases, additional clinical studies will be completed.

Footnote

[a] Giunti M, Gentilini F, Sanguinetti V, Famigli Bergamini P. Detection of canine procalcitonin in blood of SIRS dogs by means of BRAHMS PCT-Q test designed for humans. *ACVECC Postgraduate Course 2006: Sepsis in Veterinary Medicine.*

Short Answer Self Quiz Questions

1. What are the suspected etiologies for ionized hypocalcemia in human and veterinary septic patients?
2. How does ionized hypocalcemia affect mortality in human septic patients?
3. What biomarker is being evaluated for its potential role in ionized hypocalcemia in human and veterinary septic patients?
4. What are clinical signs of ionized hypocalcemia in septic patients?

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