Published online: March 30, 2020

IMR Press

Review

The dynamic relationship between serum chloride and cardiorenal syndrome

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DOI:10.31083/j.rcm.2020.01.6

Low serum sodium concentration has long been recognized as an established marker of short- and long-term morbidity and mortality in patients with heart failure (HF), and is commonly included in various risk prediction models. Mechanisms leading to hyponatremia (e.g. maladaptive neurohormonal activation) could also lead to concurrent decline in serum chloride levels. Besides, chloride has distinct biological roles (e.g. modulation of renal tubular sodium transporters) that are relevant to the pathophysiology and therapy of HF, making it a potent cardiorenal connector. Several clinical studies have recently reported on a potentially overlooked link between low serum chloride levels and adverse outcomes in patients with a wide variety of HF syndromes, which could indeed be stronger than that of sodium. While evidence on predictive value of chloride is accumulating in various patient populations and settings, the limited available interventional studies have so far yielded conflicting results. It remains to be elucidated whether hypochloremia represents a marker of disease severity and prognosis, or it is an actual pathogenetic mechanism, hence being a potential novel target of therapy. Current ongoing studies are designed to better understand the mechanistic aspects of the role of hypochloremia in HF and shed light on its clinical applicability.

Keywords

Chloride; cardiorenal syndrome; diuretics; heart failure

1. Background

Heart failure (HF) is considered a rapidly growing public health problem due to its frequency as well as its association with high rates of morbidity and mortality (Ziaeian and Fonarow, 2016). A variety of parameters have been identified as risk factors and predictors of untoward outcomes in patients with HF. Several robust predictive models of HF have so far been created and the quest continues to refine them in order to increase their predictive value. Low serum sodium is a common electrolyte disorder in this setting and extensive literature exists on the association of hyponatremia with adverse events in patients with HF. It has been shown that hyponatremia is a robust predictor of higher rehospitalization rate, prolonged length of stay, increased hospital resource utilization, and lower survival (Filippatos and Elisaf, 2013; Hauptman, 2012; Rusinaru et al., 2012). Therefore, it was anticipated that improvement in serum sodium levels through administration of vasopressin receptor antagonists (increased free water excretion through blockade of aquaporin channels in renal collecting ducts) would yield salutary impacts on the outcomes of these patients. In the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial, tolvaptan did improve dyspnea and body weight on day 1, and edema on day 7. In patients with hyponatremia, it also raised serum sodium levels significantly (Gheorghiade et al., 2007; Konstam et al., 2007). However, during a median follow-up of 9.9 months, tolvaptan did not improve cardiovascular mortality, cardiovascular death or hospitalization, and worsening heart failure compared with placebo. Besides, subgroup analysis of patients with lower serum sodium levels (>137 vs. $\leq$ 137 mEq/L) did not reveal an advantage for tolvaptan regarding those outcomes (Konstam et al., 2007).

On the other hand, since congestion is the hallmark of HF and plays a central role in its pathophysiology, dietary recommendations have conventionally focused on lowering sodium intake in HF to avoid extracellular fluid volume expansion. Nevertheless, several more recent studies have failed to show improvement in the outcomes with sodium restriction and some have even found signals of harm in certain subsets of patients (Doukky et al., 2016). Therefore, in the absence of robust studies linking low sodium intake to improved outcomes, the validity of such recommendations have been challenged and there have been calls for "a retreat from an unbridled and potentially harmful insistence on rigorous sodium restriction in those with symptomatic HF" (Yancy, 2018). To further complicate the issue, some authors have reported improved outcomes with the use of intravenous hypertonic saline for management of patients with acute decompensated HF who are hospitalized due to signs and symptoms of congestion and fluid overload (Gandhi et al., 2014; Griffin et al., 2020).

These observations have created uncertainty about the precise role of sodium in this setting and have challenged the traditional sodium-centric view that has long been dominating the field of HF. Since preservation of electroneutrality mandates presence of an anion (chloride or bicarbonate) along with sodium for homeostatic balance, investigators have recently turned their attention

to sodium's often overlooked counter-ion in salt, the chloride. Although chloride was the first electrolyte to be easily measured, it has often been overshadowed by other major serum electrolytes, seemingly serving as an adjunct to sodium or potassium or just a stand-in for bicarbonate. Herein, we provide an overview of the accumulating evidence on the role of chloride as a potent cardiorenal connector in the setting of HF.

2. Chloride homeostasis

Chloride provides about one third of extracellular tonicity (100 of the 300 mosm/L) and two-thirds of all negative charges in plasma (Koch and Taylor, 1992). It interacts with sodium to maintain serum osmolarity and fluid balance. A shift in sodium and chloride concentration triggers a fluid shift to reestablish normal solute and water ratios. In addition to its role in fluid balance that is along with sodium, chloride has an inverse relationship with bicarbonate, which is part of the key chemical buffering system responsible for preserving a normal pH. Because of its high concentration, chloride is the most important anion to maintain the balance between extracellular cations and anions to ensure electroneutrality. Intracellular chloride is highly regulated by signaling molecules that sense its changes and transduce signals to the cell membrane to modulate the conveyance of chloride transporters and channels. In particular, over the last two decades studies have uncovered the critical role of the with-no-lysine (WNK) kinases along with their upstream regulators and downstream targets (e.g. electroneutral cation-chloride cotransporters) in human physiology and disease (Berend et al., 2012; Shekarabi et al., 2017). Chloride appears to bind directly to the catalytic site of these kinases, regulating their ability to phosphorylate critical sodium-regulatory pathways. Intracellular chloride activates cation-chloride cotransporters (CCC) by promoting the phosphorylation of three highly conserved threonines in the amino terminus. The chloride-sensitive activation of CCC requires the interaction of two serine-threonine kinases, WNK3 and SPAK (a Ste20-type kinase known to interact with and phosphorylate CCC). WNK3 is positioned upstream of SPAK and appears to be the chloride-sensitive kinase. Elimination of WNK3's unique SPAK-binding motif prevents its activation of CCC. The role of chloride in the regulation of renal tubular CCC represents an important pathway that links this anion to HF. In fact, WNK acts as an intracellular sensor for chloride, and it has been shown that low intracellular chloride increases sodium-chloride cotransporter (NCC) in the distal convoluted tubules (Gamba, 2005; Giménez, 2006; Shekarabi et al., 2017). Similarly, it does increase sodium-potassium-chloride cotransporter (NKCC) activity in the thick ascending limb of the loop of Henle. Since inhibition of NCC and NKCC is the target of diuretic therapy in HF, hyperactivity of these transporters could explain the negative impact of hypochloremia on diuretic responsiveness in the clinical setting and its downstream consequences (e.g. lingering congestion).

A unique characteristic of chloride that is relevant to HF is its impact on regulation of renin secretion in the kidney, hence triggering neurohormonal activation independent of sodium. In earlier animal studies, acute and chronic administration of sodium salts other than sodium chloride failed to suppress plasma renin activity, while renin secretion was inhibited by chloride loading (with or without sodium) (Kotchen et al., 1983). Similarly, in humans, sodium chloride, but not sodium bicarbonate, could suppress renin section (Kotchen et al., 1983). Later studies revealed that in macula densa cells in the kidney, low chloride increases renin secretion through Cox-2 and prostaglandins (mainly prostaglandins I2 and E2), whereas high chloride induces adenosine secretion through undetermined mechanisms resulting in diminished renin secretion (Kotchen et al., 1987). The established role of maladaptive neurohormonal activity in the pathophysiology of HF highlights the importance of chloride concentration in the macula densa and juxtaglomerular cells in the kidney for the modulation of renin secretion.

Role of chloride on renal vasculature has been a topic of much research. Early micropuncture studies of the renal tubules suggested that hyperchloremia produces a progressive renal vasoconstriction, fall in renal blood flow, and reduction in glomerular filtration rate (GFR) that is independent of the renal nerves and seems to be specific to renal vessels (Wilcox, 1983). Moreover, it was shown that chloride availability in the renal tubules can modulate renal vasoactivity; low chloride perfusion of diminishes tubular response to vasopressors (Quilley et al., 1993). In line with those observations, more recent clinical studies have suggested that high chloride load could be associated with an increased risk of acute kidney injury (Sigmon et al., 2019; Yunos et al., 2012). Therefore, it is conceivable that various biological impacts of serum or tubular concentrations of chloride represent a less well known territory with potential clinical implications.

3. Hypochloremia and diuretic responsiveness

As discussed above, renal salt sensing is more dependent on chloride than sodium, and regulation of diuretic-sensitive sodium transporters as well as renin secretion is contingent on chloride. Since impaired renal salt excretion and aberrant sodium and water homeostasis are the key elements in pathophysiology of HF and cardiorenal syndrome, it is mechanistically conceivable that chloride could play a role as a key cardiorenal connector in this setting. There are a few clinical observations in support of this notion. In a post-hoc study on 2033 patients from PROTECT trial (Placebo controlled Randomized Study of the Selective A1 antagonist Rolofylline for Patients Hospitalized with Acute Decompensated Heart Failure and Volume Overload to Assess Treatment Effect on Congestion and Renal Function), ter Maaten et al. reported poorer diuretic response with lower serum chloride concentrations (ter Maaten et al., 2016). Hypochloremia was defined as a serum chloride level of < 96 mEq/L in this study. In order to explore the trends, patients were categorized into quintiles based on their baseline serum chloride levels, which ranged from 93.9 to 107.8 mEq/L in the first and the last quintiles respectively. They found less weight change, higher diuretic doses, smaller percentage change in B-type natriuretic peptides (BNP) levels from the baseline, and more frequent requirement for adjuvant thiazide diuretic use during hospitalization in patients with hypochloremia. Patients with low serum chloride concentration had a significantly lower rate of improvement in intravascular volume excess following therapy as indicated by poor hemoconcentration. Similarly, in a study by Hanberg et al. in an outpatient HF transitional care unit,

hypochloremia (defined as a serum chloride level of $\leq$ 96 mEq/L) was associated with higher renin levels as well as poorer diuretic response (odds ratio, 7.3; 95% confidence interval, 3.3-16.1; $P <$ 0.001) (Hanberg et al., 2016). Importantly, this association seemed to be independent of variation in serum sodium, because patients with isolated hyponatremia and normal serum chloride had comparable diuretic efficiency (i.e. mmol urine sodium per doubling of diuretic dose) to patients with normal serum sodium and chloride.

These observations are in support of chloride depletion as a candidate mechanism linking HF pathophysiology to the kidneys, and are conceivably able to affect the clinical outcomes through attenuation of the response to diuretic therapy as well as exacerbation of neurohormonal activity.

4. Hypochloremia and outcomes in heart failure

In 2015, Grodin et al. evaluated the prognostic impact of admission serum chloride in a cohort of 1318 patients hospitalized for acute decompensated HF at the Cleveland Clinic (Grodin et al., 2015). Based on their serum chloride levels, patients were categorized into tertiles; the first with a serum chloride of less than 99 mEq/L and the last with serum chloride above 103 mEq/L. They found that serum chloride levels were independently and inversely associated with long-term mortality (HR per unit change 0.93; 95% CI 0.90-0.97; $P < 0.001$) in 2261 person-years of follow up whereas admission serum sodium levels were not. In that study, they also used the data from 876 patients from University of Pennsylvania as the validation cohort, which yielded similar results (HR per unit change 0.95; 95% CI 0.92-0.99; $P = 0.01$). Based on their serum chloride levels, patients were categorized into 3 groups; lower than 99, 99 to 103, and greater than 103 mEq/L. The interesting finding of this study, which was reproduced later by other authors, was that the prognostic value of sodium was diminished or disappeared in the face of hypochloremia. Indeed, hyponatremia did not seem to significantly affect the outcomes if accompanied by normal serum chloride levels while there was a correlation between hypochloremia and mortality even when serum sodium was normal, implying a stronger prognostic value for serum chloride levels.

A year later, the same concept was evaluated in a cohort of 1673 patients with stable chronic HF (Grodin et al., 2016). In order to explore the trends, patients were categorized into quartiles based on their baseline serum chloride levels, which ranged from less than 101 to more than 104 mEq/L in the first and the last quartiles respectively. Over a 5-year follow up period, the investigators found that lower serum chloride levels were independently and incrementally associated with increased mortality risk; after extensive adjustment, every standard deviation decrement in chloride (i.e. 4.1 mEq/L) was associated with a 26% increased risk of 5-year mortality (HR 1.26, 95% CI 1.03-1.55; $P = 0$ .03). Interestingly, similar to ADHF population, low serum sodium levels were not found to be associated with mortality after adjustment for chloride. Subgroup analyses revealed that lower chloride levels had consistent risk of mortality across a number of dichotomized subgroups (e.g. eGFR $<$ or $\geq$ 60 mL/min per 1.73m^2). The fact that serum chloride levels were strongly correlated to functional status, blood urea nitrogen, and loop diuretic use, while they were not closely correlated with more traditional markers of HF severity, such as LVEF and BNP, suggested an extracardiac link between aberrant chloride homeostasis and these parameters (Grodin et al., 2016).

These results were reproduced in a Chinese population where the investigators found an inverse association between serum chloride levels with mortality in chronic HF and suggested a role for it in increasing the prognostic value of serum sodium levels (Zhang et al., 2018). A study from the UK included both patients with reduced and preserved ejection fraction (Cuthbert et al., 2018) and showed that low serum chloride is associated with increased risk of adverse outcomes regardless of HF phenotype and independent of conventional prognostic variables such as NT-proBNP levels. Hypochloremia was defined as serum chloride $<$ 96 mEq/L, and the patients were categorized into 6 groups based on their baseline serum chloride levels which ranged from 94-98 to 105-107 mEq/L. The study also reported a potential association between hypochloremia and sudden death in HF. Since NKCC and NCC have a role in maintaining myocyte volume and pH, it is plausible that dysregulation of these transporters due to hypochloremia could be contributing to arrythmogenicity and impaired myocyte contractility (Adkins and Curtis, 2015; Clemo et al., 1992). Several other studies exploring the role of chloride in HF have reported similar findings (Grodin et al., 2017, 2018; Hanberg et al., 2016; ter Maaten et al., 2016). It should be noted that the majority of these studies are retrospective or unplanned post-hoc data analysis of trials in which the changes in the level of serum chloride were not the study question or endpoint. As such, while the association of low chloride with adverse outcomes seem to be consistent among various patient populations, a potential causal relationship needs to be explored by future prospective studies.

5. Clinical impact of increasing serum chloride level

In order to further elucidate a potential causal link between hypochloremia, diuretic resistance, and adverse outcomes, it may be helpful to raise serum chloride levels and study its downstream biological effects and clinical consequences. The increase in serum chloride can be achieved either through simple chloride supplementation or via pharmacologic agents. Chloride supplementation is not a novel approach and has indeed been known since the 1960's to improve diuretic responsiveness and portend a salutary effect on treatment of refractory fluid retention (Rubin et al., 1960). More recently, in a proof of concept study, oral lysine chloride supplementation was used for three days in 10 stable patients with HF (mean left ventricular ejection fraction of 37.3%) and hypochloremia (Hanberg et al., 2016). While supplementation did increase serum chloride levels, it paradoxically raised plasma renin activity too without changing total urine volume; there was even a trend toward worsening urine output in patients with high pre-supplementation urine volume. The observed changes in the available metrics and markers of volume status (i.e. weight, NT-pro-BNP, serum albumin, and BUN/creatinine ratio) implied intravascular volume contraction following chloride supplementation. While there was no significant change in serum creatinine, cystatin C, or the kidney injury biomarkers (i.e. neutrophil gelatinase-associated lipocalin, kidney injury molecule-1,

and interleukin-18), the blood urea nitrogen to creatinine ratio increased by 22%, implying development of intravascular volume contraction. Acetazolamide has been tried as a pharmacological method of raising serum chloride levels in HF. There have been concerns about development of tachyphylaxis with long-term acetazolamide use as well as untoward consequences (e.g. metabolic acidosis and hypokalemia), which could limit its clinical applicability in the setting of chronic HF. Kataoka (2019) investigated the prolonged (i.e. 11-60 days) administration of acetazolamide in patients with HF, and reported that it was efficient at maintaining the rise in the serum chloride level and was well tolerated. The Diamox to Increase the Urinary Excretion of Sodium: an Investigational Study in Congestive Heart Failure (DIURESIS-CHF) trial was a randomized controlled study exploring the impact of acetazolamide use on natriuresis, decongestion, and neurohumoral activation in 34 patients with acute HF (mean left ventricular ejection fraction of 43%) and fluid overload who were at high risk for development of diuretic resistance (Verbrugge et al., 2019). In comparison with high dose loop diuretic therapy, addition of acetazolamide to low dose loop diuretics was associated with similar sodium excretion and decongestion, hence increasing loop diuretic efficiency. Changes in neurohormonal activation, natriuretic peptides, and serum potassium concentrations were comparable in both arms, and there was a non-significant trend towards lower all-cause mortality or heart failure readmissions in the group receiving acetazolamide. Five patients in the combinational treatment arm with acetazolamide (28%) vs. none in the high-dose loop diuretic monotherapy arm developed rise in serum creatinine of $>$ 0.3 mg/dL within 72 hours (P = 0.046). It is noteworthy that the clinical implication and prognostic value of changes in serum creatinine following therapy of HF have been a matter of much controversy in recent years, especially if the rise in serum creatinine level is associated with efficient decongestion (Kazory and Ronco, 2019).

A recent meta-analysis including 9 studies with a total number of 229 patients with HF reported that acetazolamide decreased serum bicarbonate and PH levels and improved apnea indices (Wongboonsin et al., 2019). Compared to placebo, acetazolamide could also significantly increase urinary sodium excretion. While these observations are limited in quantity and quality, their promising results have renewed interest in the use of this old diuretic and call for future more robust studies to further elucidate its role in this setting.

ADVOR (Acetazolamide in Decompensated Heart Failure with Volume Overload) is an ongoing large-scale multicenter randomized controlled trial in Belgium that explores the impact of daily use of acetazolamide vs. placebo on a background therapy of high-dose loop diuretics in patients with acute decompensated HF (Mullens et al., 2018). Another randomized controlled trial is currently ongoing where several clinical and laboratory parameters (e.g. blood volume and renal function) are monitored in patients with HF who receive 7 days of therapy with lysine chloride or placebo (ClinicalTrials.gov-NCT03446651).

6. Conclusion

Chloride has recently been identified as a potential cardiorenal connector in the setting of HF. Its distinct biological activities can explain pathophysiologic pathways (i.e. neurohormonal activation and enhancement of tubular sodium transporters) through which it can portend its effects. Clinically, several studies have reported on a link between low serum chloride levels and adverse outcomes in patients with a wide variety of HF syndromes. While evidence on predictive value of chloride seems to be accumulating in various patient populations and settings, the limited available interventional studies in this field have so far yielded conflicting results. It remains to be elucidated whether chloride represents a marker of severity and prognosis of the disease and its underlying pathophysiology, or it portends a causal relationship, hence being a novel target of therapy.

We need to be aware that the great majority of these data are generated either from retrospective studies or from unplanned post-hoc data analysis of trials in which the changes in the level of serum chloride were not the study question or the primary or secondary endpoint. Therefore, while highly consistent, they are fraught with inherent limitations of such analysis. Moreover, chloride is known to be involved in acid-based homeostasis and is tightly linked to changes in pH. As such, future studies are needed to determine whether changes in pH could modulate the association of hypochloremia and outcomes such as diuretic responsiveness. In view of these findings, it will be prudent to mandate all future HF therapy trials (e.g. those on extracorporeal ultrafiltration) to examine the impact on serum chloride levels and include it as a safety endpoint. Indeed, there should be a demand for reporting of the changes in chloride in such studies as it can provide some insight into the impact on the outcomes. Finally, the contemporary risk prediction models of HF have typically included sodium, hemoglobin, and BUN in their analyses. In view of the accumulating evidence on the strong predictive value of hypochloremia in a wide range of HF syndromes, it needs to be verified where incorporation of serum chloride level into these models would add to their predictive value.

Authors' contributions

Amir Kazory: Drafting article and data collection; Maria Rosa Costanzo: Revising the article critically for important intellectual content

Acknowledgment

Thanks to all the peer reviewers and editors for their opinions and suggestions.

Conflict of interest

No specific financial support was obtained for preparation of this article. The authors declare no conflicts of interest statement.

Submitted: January 27, 2020
Accepted: March 25, 2020
Published: March 30, 2020

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