Effect of Hyponatremia and its Normalization on Patients' Clinical Outcomes and Bone Metabolism

Inaugural Dissertation

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by

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Approved by the Faculty of Medicine

On application of

Professor Dr. med. Mirjam Christ-Crain (first supervisor)

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Basel, ……

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Professor Dr. med. Primo Schär, Dean

Schriftliche Erklärung

Ich erkläre, dass ich die Dissertation

Effect of Hyponatremia and its Normalization on Patients' Clinical Outcomes and Bone Metabolism

nur mit der darin angegebenen Hilfe verfasst und bei keiner anderen Universität und keiner anderen Fakultät der Universität Basel eingereicht habe.

Ich bin mir bewusst, dass eine unwahre Erklärung rechtliche Folgen haben kann.

Basel, 29.11.2021

(Ort, Datum)

(Unterschrift)

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Acknowledgment

This was almost the most difficult part to write, despite the fact that I am very thankful to my friends and colleagues, and of course my family and my partner, for having supported, encouraged and taught me a lot in the past years. This project would have never been possible without my *Chefin* and mentor Prof. Mirjam Christ-Crain, a very competent and inspiring woman, and her working group. Especially Dr. Julie Refardt and Dr. Clara Sailer had supported me a lot during these 3 years, as well as our study nurses Joyce dos Santos, Cemile Bathelt and Nina Hutter, and all other colleagues working under the supervision of Prof. Marc Donath. And of course this project owed a lot to Professor Christian Meier, my second supervisor. With its expertise and enthusiasm, he let my interest in bone metabolism grown up. I thank my family and friends, who sustain me despite the distance, which I had noticed anyway only in terms of kilometers. And I thank my partner, Dr. Björn Frye, for the caring daily all-round encouragement and support, in working and private life.

Questa è stata quasi la parte più difficile da scrivere, anche se non dipende dal fatto che io abbia dubbi sulle persone che hanno reso possibile che io arrivassi fin qui. E sono tante, cominciando dalla mia capa e mentore Professoressa Mirjam Christ-Crain, che con la sua competenza e fiducia è continuamente di forte inspirazione. E sono grata ai miei colleghi e colleghe, con particolare minzione per le dottoresse Julie Refardt e Clara Sailer, che mi hanno aiutata a tantissimo in questi 3 anni, e alle nostre study-nurse Joyce Dos Santos, Cemile Bathelt e Nina Hutter, perché senza il loro prezioso aiuto sarebbe difficile portare a termine gli studi clinici, e tutti i colleghi e colleghe che hanno o hanno avuto la fortuna come me di lavorare nel reparto del Professore Marc Donath. Inoltre, questa tesi deve molto al professore Christian Meier, mio secondo supervisore, perché con il suo entusiasmo e la sua competenza ha fatto crescere il mio interesse per il metabolismo osseo.

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Summary of the project

Background: Hyponatremia is an electrolyte disorder affecting up to 40% of hospitalized patients, and has widely been associated with increased risk of death, rehospitalizations, as well as with falls, fractures and osteoporosis, when present on admission. Despite this association, knowledge about beneficial effects of its correction is scare. Additionally, hyponatremia is mostly considered as a surrogate of disease severity rather than a treatable trait, resulting in many patients being and / or remaining hyponatremic at discharge.

Objective: This MD-PhD thesis investigates the hypothesis that correction of hyponatremia in hospitalized, adult patients improves their clinical outcome, with particular focus on bone metabolism.

Methods: The first study is a secondary analysis of a prospective, placebo-controlled trial to understand the impact of hyponatremia at discharge on clinical outcomes in hospitalized patients with pneumonia. The second study is a registry analysis to investigate the impact of persistency versus normalization of admission hyponatremia on clinical outcomes in hospitalized patients with stroke. The third and main study is the analysis of plasma bone marker changes in relation to serum sodium levels during a prospective, placebo-controlled interventional trial in hospitalized patients with syndrome of inappropriate antidiuresis- (SIAD) induced hyponatremia.

Results: Overall, all three studies support the hypothesis that in-hospital correction of hyponatriemia improves the outcome of affected patients. In the setting of pneumonia, hyponatremia at discharge is associated with increased risk of a recurrence within 6 months. In patients with stroke, persistency of initial hyponatremia is associated with a worse functional outcome at 3 months. In patients with SIAD, a targeted correction of hyponatremia stimulates bone formation.

Discussion: Newly developed or persistent hyponatremia at discharge is associated with worse clinical outcomes in hospitalized patients. Correction of hyponatremia reverses the hyponatremia-induced negative effect on bone metabolism by stimulating bone formation. Further interventional studies are needed to clarify whether correction of hyponatremia could

improve other hyponatremia associated patients' relevant clinical outcomes analogue to improving bone metabolism as well as long-term of this effect.

Zusammenfassung des Projektes

Hintergrund: Hyponatriämie ist eine häufige Elektrolytstörung mit einer Prävalenz bis zu 40% in hospitalisierten Patienten. Eine bei stationärer Aufnahme bestehende Hyponatriämie wurde weithin mit einem erhöhten Sterberisiko, Re-Hospitalisierungen sowie mit Stürzen, Frakturen und Osteoporose in Verbindung gebracht. Trotz dieser Assoziation ist nur sehr wenig über die Auswirkungen einer Korrektur der Hyponatriämie auf relevanten klinischen Ergebnissen bekannt. Darüber hinaus wird Hyponatriämie meistens als Krankheitsmarker und nicht als behandelbare Erkrankung angesehen, sodass eine Korrektur einer bestehenden Hyponatriämie ausbleibt und Patient*innen hyponatriäm entlassen werden.

Ziel: Diese Doktorarbeit zielt darauf ab, die Auswirkungen der Korrektur der Hyponatriämie gegenüber der Präsenz oder Persistenz der Hyponatriämie bei Austritt auf relevanten klinischen Ergebnisse bei hospitalisierten erwachsenen Patienten zu untersuchen, mit besonderem Fokus auf den Knochenstoffwechsel.

Methoden: Das erste Projekt ist eine Sekundäranalyse einer prospektiven, placebokontrollierten Studie zum Verständnis der Auswirkung einer Hyponatriämie bei Austritt auf klinischen Ergebnissen bei hospitalisierten Patienten mit Pneumonie. Das zweite Projekt ist eine Registeranalyse, um den Einfluss der Persistenz gegenüber der Normalisierung der Eintrittshyponatriämie auf klinischen Ergebnissen bei hospitalisierten Patienten mit Schlaganfall zu untersuchen. Das dritte und Hauptprojekt ist die Analyse von Veränderungen der Plasma-Knochenmarker in Bezug auf Serumnatriumspiegel während einer prospektiven, placebokontrollierten Interventionsstudie bei hospitalisierten Patienten mit Syndrom der inadäquaten Antidiurese (SIAD) induzierter Hyponatriämie.

Ergebnisse: Insgesamt stützen alle drei Studien die Hypothese, dass die Korrektur der Hyponatriämie in hospitalisierten Patienten deren Outcome verbessert. Eine Hyponatriämie bei Austritt bei Patienten mit Pneumonie ist mit einem erhöhten Rezidivrisiko innerhalb von 6 Monaten verbunden. Die Persistenz der initialen Hyponatriämie in hospitalisierten Patienten mit Schlaganfall ist mit einem schlechteren funktionellen Ergebnis nach 3 Monaten verbunden.

Eine gezielte Korrektur der Hyponatriämie bei hospitalisierten Patienten mit SIAD-induzierter Hyponatriämie stimuliert die Knochenbildung.

Diskussion: Eine bis zur Entlassung persistierende oder neu entstehende Hyponatriämie erhöht bei hospitalisierten Patienten das Risiko für einen schlechteren klinischen Outcome. Die Korrektur der Hyponatriämie setzt die durch Hyponatriämie induzierte negative Wirkung auf den Knochenstoffwechsel durch Stimulierung der Knochenbildung zurück. Interventionsstudien sind erforderlich, um zu klären, ob eine Korrektur der Hyponatriämie weitere relevanten klinischen, mit Hyponatriämie assoziierten Ergebnisse verbessern könnte.

Introduction

Hyponatremia, defined as a serum sodium concentration below 135 mmol/l, is the most common electrolyte disorder in hospitalised patients, with a prevalence between 17 and 30% in adults and up to 40% in elderly patients[1].

Hyponatremia is defined as acute when it occurs within the previous 48 hours, otherwise it is defined as chronic[2].

The Pathophysiology of Hyponatremia

The definition of hyponatremia as a decreased serum sodium concentration implies a relative water excess in extracellular fluid volume (ECF) as compared to sodium. Hyponatremia is therefore the result of a disturbance in water balance. Sodium, urea and glucose determinate serum concentration, defined as serum osmolality[3]. The relative excess of water in hyponatremia lowers serum osmolality, altering the homeostasis between cells and ECF. As water flows from a compartment with low osmolality to a compartment with high osmolality, cells internalize water till a new osmotic equilibrium is reached. In other words, hyponatremia causes cells swelling[4]. In addition, receptors for osmolality and blood volume status, called osmo- and baroreceptors, trigger an interaction between brain and kidneys to maintain water balance. The hormone arginine-vasopressin (AVP) plays an important role in this interaction. Of note, regulation of water balance is primarily driven by control of serum osmolality, and only secondarily by blood volume status[5] [Hoorn 2008].

Physiologically, when serum osmolality rises up, cells in the supraoptic nuclei of the hypothalamus change their volume, triggering a cascade of events that brings to secretion of AVP. Similarly, when effective circulating blood volume decreases, carotis sinus baroreceptors send a signal to the paraventricular nuclei of hypothalamus causing release of AVP[6].

AVP induces the insertion of aquaporin water channels in the apical membrane of the kidneys and increases the number of epithelium sodium channel ENaC and of the urea transporter UT-A1, increasing water reabsorption both directly through aquaporin water channels and

indirectly through sodium and urea reabsorption, reestablishing the balance in serum osmolality and/or effective circulating blood volume[5]. Figure 1 displays AVP release in respond to physiological stimuli.

Figure 1 – Physiological regulation of AVP release

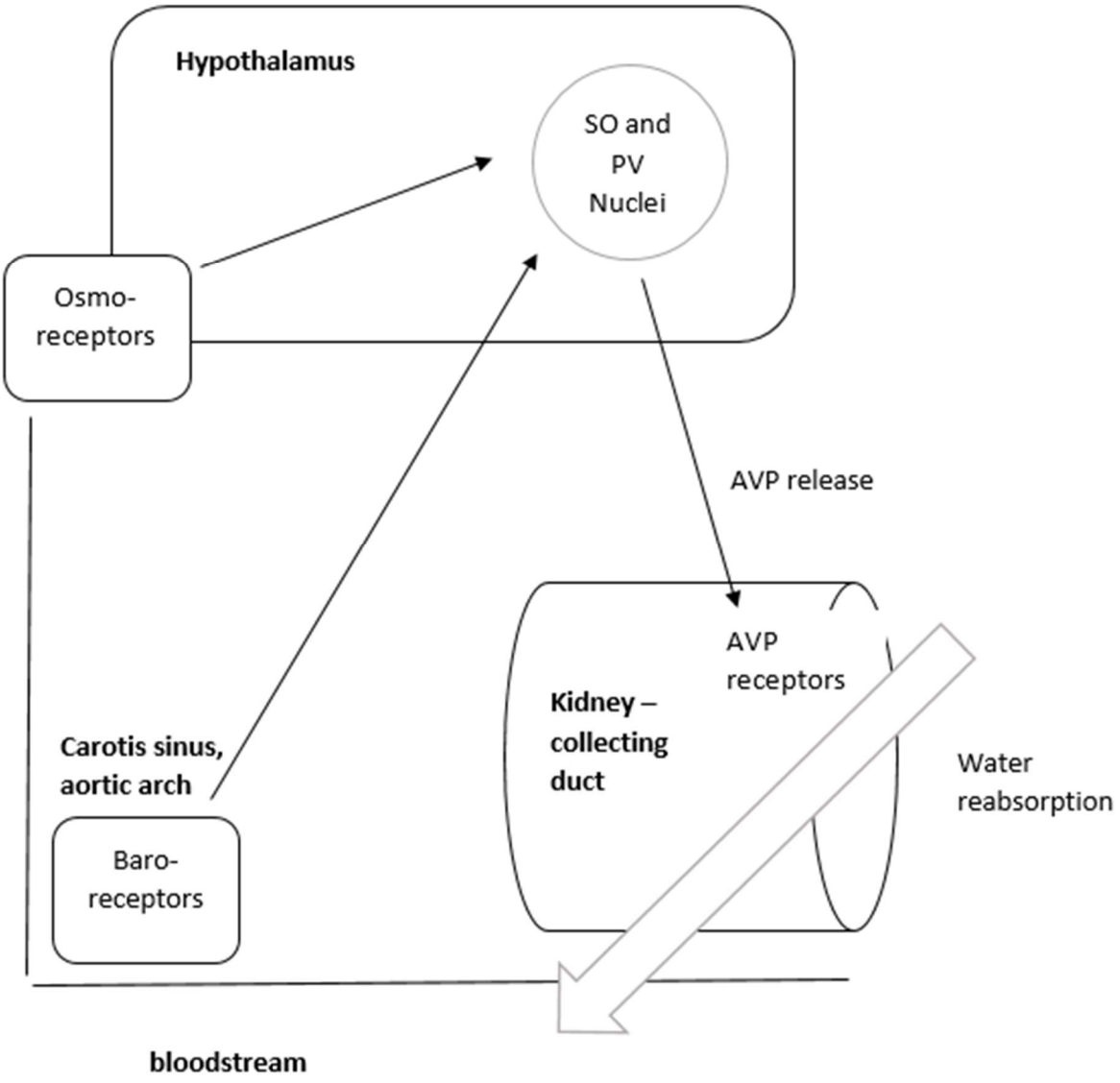


Figure 1 - Arginine-vasopressin (AVP) is produced in the hypothalamus and released in bloodstream by the posterior pituitary gland in respond to osmotic stimuli or reduction of circulating blood volume. Activation of AVP receptors in the collection duct of the kidney results in water absorption.

Hyponatremia results from an inappropriate AVP release (i.e. despite a lack of osmotic or barometric stimulus like in case of ectopic, paraneoplastic AVP production, or by high interleukin-6-levels) or from circumstances mimicking AVP-release (e.g. drugs mimicking AVP effects or activating mutations in the vasopressin receptors)[7–10].

Notably, hyponatremia and the corresponding relative water excess or, in other words, relative lack of sodium, can occur in hypervolemia (expanded ECF), but also in case of euvolemia (normal ECF), or even hypovolemia (contracted ECF)[5,11,12]. For example, a secondary adrenal insufficiency stimulates AVP production causing hyponatremia due to the corticotropin-releasing hormone (CRH) increase, which is independent of the volume status and may happen in a context of euvolemia (normal ECF). In case of a primary adrenal insufficiency, AVP production is additionally induced by the reduced renal ability to reabsorb sodium, which leads to water loss and hypovolemia (contracted ECF) in the context of hyponatremia[5,11,12].

Hyponatremia symptoms

Hyponatremia is graded according to serum sodium concentration in mild (serum sodium concentration 130 – 134 mmol/l), moderate (serum sodium concentration 125-129 mmol/l) and profound (serum sodium concentration <125 mmol/l)[13]. Clinically, hyponatremia is denoted as "severe" if it is associated with symptoms like strong headache, nausea, vomiting, confusion, seizures or coma. Pathophysiologically, this is the result from cell swelling caused by lowered serum osmolality. In contrast, rapid correction of hyponatremia can result in cellular shrinking and therefore osmotic demyelinization[2,14].

Chronic, stable hyponatremia is often considered clinically asymptomatic, resulting in clinical neglect. However, in the past years many different studies have suggested a strong association between hyponatremia and increased mortality and morbidity such as gait instability and cognitive impairment, independent from underlying disease[14].

This brought a raising awareness of hyponatremia not only as a surrogate of disease severity but also as an independent factor influencing morbidity and mortality beyond underlying

disease. In other words, adaption to hyponatremia may be clinically unperceived, but it seems anyway to alter body homeostasis in a remarkable way[5].

Association between hyponatremia and worse patients' outcome

The awareness of a possible association between hyponatremia and worse patients' outcome started to rise up almost forty years ago. Already in 1986 Tierney et al.[15] performed an analysis in 13,979 hospitalized patients comparing matched cohorts of patients with and without hyponatremia, and described a strong association with in- and out-hospital mortality (p less than 0.0001 for both) regardless of the underlying disease. More recently, Wald et al.[16] confirmed these results in a cohort of 53,236 patients. In their study, hyponatremia on admission was associated with an adjusted odds ratio of 1.52 for in-hospital mortality, of 1.12 for being discharged in a short- or long-term care facility, and a 14% longer hospitalization time, independent from underlying disease. Another observational study in 2018 analyzing 2.3 million hospitalized patients concluded that hyponatremia is significantly associated with in-hospital mortality, and discharge to hospice or to a nursing facility[17].

These findings of worse outcome in hyponatremic patients have been shown in several cohorts with different underlying conditions like older age[18], acute medical conditions[19], cancer[20], kidney failure[21], chronic obstructive pulmonary disease[22] (COPD), subarachnoid hemorrhage[23], liver cirrhosis[24], heart failure[25], myocardial infarction[26], pneumonia[27], stroke[28], and more recently COVID-19[10,29], and confirmed by different meta-analysis[30,31].

All these studies emphasize an association between hyponatremia and worse outcome independent from underlying disease, but could not verify a causal relation. Therefore, it is not known whether correction of hyponatremia might improve patients' outcome.

An important contribute in this sense was given by a case report from 2014[32] [Sejling 2014]. The authors described how a young man recovered from osteoporosis after resolution of hyponatremia due to an AVP producing tumor. The young man was suffering from osteoporosis since many years and antiresorptive therapy was not giving any relief. This case

report pointed out to clinical implications of chronic hyponatremia and its correction on bone health.

Hyponatremia and bones

Since many decades, different studies reported an association between hyponatremia and changes in bone health. Analogously to the association with mortality, rehospitalization, length of hospitalization and discharge destinations, many different observational studies reported that patients with hyponatremia have an increased risk of falls, fractures, and osteoporosis[33–35]. A population-based analysis showed that patients with hyponatremia have an odds ratio for fractures of 4.61 (95% CI, 4.15–5.11)[36]. Moreover, patients with a documented hyponatremia in the last 30 days have an odds ratio of 3.05 (95% CI, 2.83–3.29), and patients with a documented hyponatremia in the last 12 months of 11.21 (95% CI, 8.81–14.26)[36].

In addition, there is evidence that orthopedic injury might represent the first manifestation of chronic hyponatremia in the elderly[37]. According to Ayus et al., hyponatremia on admission was present in 20% of elderly patients with hip fractures. The same authors suggest to add serum sodium levels in the Fracture Risk Assessment Tool (FRAX®)[38].

As opposed to the association between hyponatremia and clinical outcomes, many in vitro experiments as well as experiments on animal models are available for the association between hyponatremia and changes in bone metabolism, especially from the working group of doctor (dr.) Joseph G. Verbalis. In a rat model, Verbalis et al. could histologically show a thinner cortical and trabecular bone with increased number of activated osteoclasts in mice with hyponatremia when compared to normonatremic controls[39]. Moreover, it has been shown that hyponatremia induces a reduction of osteoblasts by switching the differentiation of mesenchymal cells towards adipocytes rather than osteoblasts and by activating osteoclasts[40].

While all these studies agree that changes in serum osmolality do not play a role in hyponatremia induced bone changes, the role of changes in levels of circulating AVP is controversial. Verbalis et al. in 2010 showed that the effect of hyponatremia on bone is merely driven by serum sodium levels, whereas changes in levels of circulating AVP do not affect

bone structures[39]. In their rat models, they showed that osteoclasts were increased in rats with liquid load but not in rats with solid diet, although both of rats-groups had an increased AVP through intravenous infusion of desmopressin, an agonist of the AVP receptor *Avpr2*[39]. In contrast, Tamma et al.[41] showed in a mice model a direct effect of AVP on osteoblasts and osteoclasts. In their experiment, they identified two receptors for AVP, *Avpr1α* and *Avpr2*, both present on osteoblasts and osteoclasts. After injection of AVP, mice with a genetic deletion of *Avpr1α* had an increase in osteoblastogenesis, whereas wild-type mice had an increase in osteoclastogenesis[41]. The same working group confirmed these results in 2016 in another mice model[42], showing that *Avpr1α* and the oxytocin receptor *Oxtr* have opposing effects on bone mass. The authors concluded that the incongruence with the results of Verbalis et al. might be caused by the use of a *Avpr2* agonist with no effect on *Avpr1a* by the working group of Verbalis.

Biomarkers for bone resorption and bone formation

In humans, it is difficult to perform histological studies to investigate the effect of hyponatremia and its correction on bone health, as it would implicate to perform invasive bone biopsies. In the past decades, an important progress has been made in the identification of specific bone biomarkers (BTMs), products of bone remodeling that can be detected in blood or urine. These markers have originally been developed to aid the management of metabolic bone disease, and are slowly endorsing the role of a resource for non-invasive assessment of bone metabolism in different pathological states, including hyponatremia[43–45].

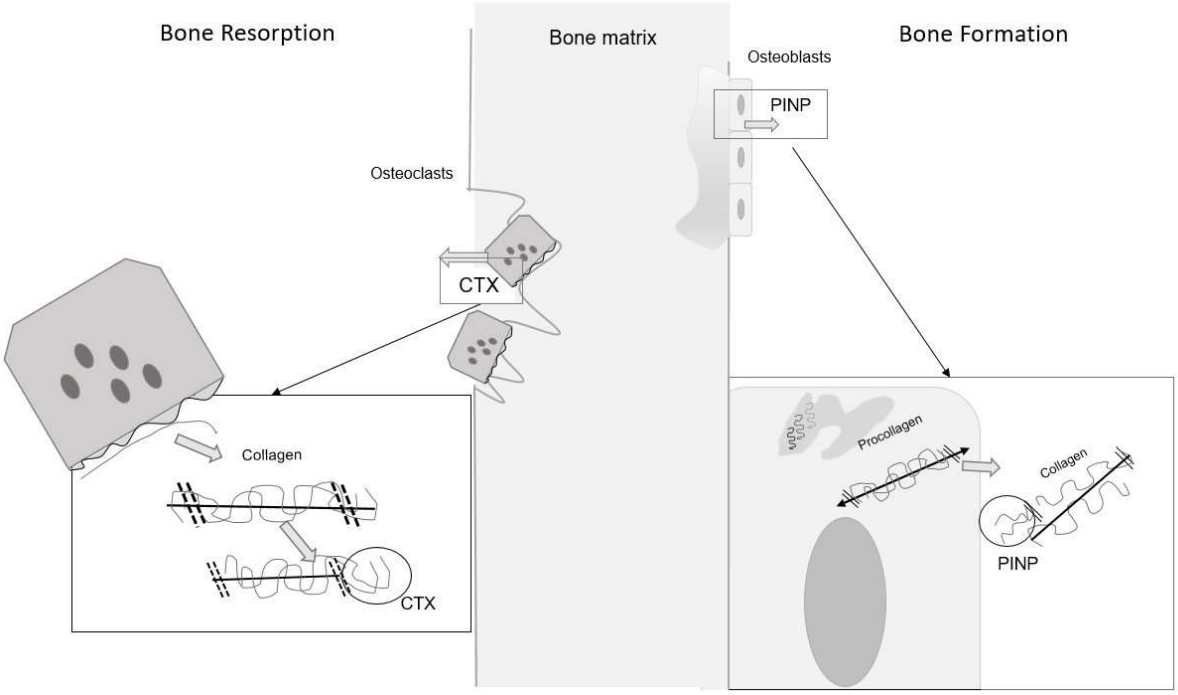
The main components of bone are crossed linked type-1 collagen and hydroxyapatite. Many different factors are involved in bone remodeling (parathyroid hormone PTH, vitamin D, paracrine molecules released by osteocytes etc.), a process that lasts about 200 days and implies a balance between bone formation and bone resorption. Although osteoblasts are mainly involved in bone formation and osteoclasts in bone resorption, these cells interact strictly with each other and their balance is very central to maintain bone homeostasis. Several BTMs have been identified, both produced during bone formation and bone resorption, but in

the last years two BTMs have reached the highest consensus for representing bone formation and bone resorption, procollagen type I N propeptide (PINP) and Carboxy-terminal cross-linked telopeptides of type-1 collagen (CTX) (Figure 1)[43,46,47].

PINP is a BTM measurable in blood as a marker for bone formation. During bone formation, osteoblasts produce type-1 collagen precursor procollagen 1 and secrete it in the bone matrix, where it is digested in type-1 collagen. PINP is one of the cleavage products of procollagen 1, produced during extracellular processing of procollagen-1. Different studies have shown that PINP levels correlate significantly with histomorphometric measures of bone formation, as the amount of produced PINP is the same of the amount of collagen incorporated into bone matrix[48,49]. However, caution is needed in case of rapid growth and fibrotic processes, as PINP is also synthesized in skin and other connective tissues. Moreover, caution is needed in patients with impairment of liver function, as PINP is metabolized by the liver. PINP has been recommended by the Bone Marker Standards Working Group because of its low inter-individual variability, the stability in serum at room temperature, its minimal circadian rhythm and the fact that is value is not altered by food intake[43].

CTX is a BTM measurable in blood as a marker for bone resorption[50,51]. Bone resorption occurs in respond to osteoclast factors that induce digestion of type-1 collagen. CTX is one of the cleavage products of type-1 collagen. CTX production follows a circadian rhythm, moreover, CTX is less stable after blood sampling and is more influenced by food intake than PINP. In addition, caution is needed in patients with kidney failure, as CTX is cleared by the kidneys. For all these reasons, especially by CTX measurements particular attention must be taken to possible pre-analytic biases[43,52].

Figure 2 - Release of specific bone biomarkers during bone formation and bone resorption



During osteoblast mediated bone formation, the procollagen I is digested in type 1 collagen. Procollagen type I N propeptide (PINP) is released in the bloodstream as a cleavage product of this digestion. During osteoclast mediated bone resorption collagen I is degraded, releasing cross-linked C-telopeptide (CTX) in the bloodstream.

Due to possible pre-analytical problems and to the challenge of developing specific and reliable essays, BTMs have only recently been used for clinical research purposes.

In 2020, an analysis of hospitalized patients with acute hyponatremia showed that acute mild hyponatremia is associated with a reduction of bone formation defined as PINP/CTX ratio[53]. Moreover, an exploratory secondary analysis comparing the effect of tolvaptan versus placebo investigated the effect of an intervention for correction of hyponatremia on bone markers in humans, showing a possible effect both on osteoblast and osteoclast activation[54]. In their analysis, Verbalis et al. investigated the effect of vaptans on bone resorption index, defined as change from baseline in urine NTx-creatinine ratio (a bone resorption marker) divided by serum osteocalcin concentration (a bone formation marker). This was also the first study investigating

the possible impact of correction of hyponatremia on bone metabolism. However, due to the limited availability and the preliminary nature of the data, the clinical impact of correction of hyponatremia on bone metabolism still needs to be clarified.

Altogether, despite the wide knowledge about in-hospital hyponatremia and impairment of patients' relevant clinical outcomes and bone health, very little is known about the possible impact of correction of hyponatremia on patients' outcomes. This lack of evidence is mirrored by the limited awareness of hyponatremia in clinical practice, with about 50% to 80% of hospitalized patients with hyponatremia on admission being still hyponatremic at discharge[1,55].

The goal of this PhD-Project was therefore to investigate whether normalization versus persistency of hyponatremia have a positive impact on outcome beyond mortality of hospitalized patients with pneumonia and stroke, and to assess changes in bone metabolism after correction of hyponatremia. This provided new knowledge can support correction of hyponatremia in hospitalized patients as being an important therapeutic intervention to improve patients' outcomes.

Main objectives of this MD-PhD

Based on the above, these are the main following objectives for this PhD-project

Manuscript 1

Hypothesis: Not only hyponatremia on admission, but also hyponatremia at discharge might contribute to a worse clinical outcome in hospitalized patients with pneumonia.

Objective: To investigate whether hyponatremia at discharge is associated with increased risk of death, rehospitalization, or recurrence at 6 months in hospitalized patients with community-acquired pneumonia.

Manuscript 2

Hypothesis: Persistency of initial hyponatremia in hospitalized patients with stroke negatively influences patients' relevant clinical outcomes.

Objective: To investigate in stroke patients whether persistency of initial hyponatremia affects patients' disability measured by modified Rankin Scale at three months; mortality and stroke recurrence within three months; length of hospitalization; and discharge destination, as compared to normonatremia, in-hospital acquired hyponatremia, and normalization of initial hyponatremia.

Manuscript 3

Hypothesis: A targeted correction of hyponatremia positively influences bone metabolism reversing the hyponatremia-induced changes.

Objective: To investigate whether and how correction of hyponatremia changes CTX, PINP and their ratio in hospitalized patients with SIAD-induced hyponatremia.

Contribution by the MD-PhD student

Manuscript 1

- Exhaustive literature research on hyponatremia and pneumonia
- Formulation of clinical question
- Data analysis
- Discussion of the results with primary and secondary supervisor
- Writing of the manuscript
- Manuscript submission
- Writing of rebuttal letter to objections raised by reviewers
- Manuscript publication process

The first manuscript was published as a shared first authorship with Dr. Clara Sailer and Dr. Claudine Blum. Dr. Clara Sailer was involved in the recruitment of the patients and helped with the statistical analysis as well as in the writing process of the manuscript. Dr. Claudine Blum was ideating the placebo controlled, prospective trial at the basis of this analysis and helped in the writing process of the manuscript.

Manuscript 2

- Comprehensive literature research on hyponatremia and stroke
- Meeting with supervisors and other experts to formulate a clinical question
- Several discussions with supervisors and other experts to develop an appropriate study design to answer the clinical question
- Successful application for a Research Grant awarded by the University of Basel (“Wissenschaftspool”)
- Writing of the protocol
- Preparation of necessary documents for submission of the trial to the competent ethics committee (CEC)
- Correspondence with the CEC

- Supervision of matching data extracted from Swiss Stroke Registry with clinical data extracted from the electronic patient records
- Data analysis in collaboration with the Clinical Trial Unit of University Hospital of Basel
- Writing of the manuscript
- Manuscript submission
- Writing of rebuttal letter to objections raised by reviewers
- Manuscript publication process

Manuscript 3

- A detailed literature research on the impact of hyponatremia on bone metabolism, fractures and osteoporosis, in clinical studies as well as in histological and animal models.
- Formulation of the clinical question
- Several meetings with supervisors to develop an appropriate work-up to answer the clinical question
- Participation in placebo-controlled trial: Patient recruitment by active screening of medical records at the University Hospital Basel; study visits conduct
- Sample shipment to special laboratory for measuring bone markers
- Data analysis
- Discussion of the results with primary and secondary supervisor
- Writing of the manuscript
- Manuscript submission
- Writing of rebuttal letter to objections raised by reviewers
- (Manuscript publication process)

The third manuscript was published as a shared first authorship with Dr. Julie Refardt. Dr. Julie Refardt was ideating the placebo controlled, prospective trial at the basis of this analysis and helped with the formulation of the clinical question as well as in the writing process of the manuscript.

Moreover, I am co-investigator in a still ongoing multicentric study on 2278 hospitalized patients investigating the effect of a targeted correction of hyponatremia versus standard care on mortality and rehospitalization in hospitalized patients with no hypertonic hyponatremia (EKNZ 2018-00971, NCT 03557957)[56], organizing the initiation of 4 Swiss centers and 4 international centers, and performing recruitment and intervention.

Manuscript 1: Mild to moderate hyponatremia at discharge is associated with increased risk of recurrence in patients with community-acquired pneumonia

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Abstract

Background

Hyponatremia is the most common electrolyte disorder in hospitalized patients with pneumonia. Different studies have shown an association of hyponatremia on admission and worse patient's outcome. Yet, the impact of hyponatremia at discharge or of hyponatremia correction on patient's prognosis is unknown.

Methods

This is a preplanned secondary data analysis from a double-blind, randomized, placebo-controlled trial of hospitalized patients with community-acquired pneumonia and prednisone treatment. The primary outcome was the impact of hyponatremia on admission and at discharge on patient relevant outcomes (i.e. mortality, rehospitalization and recurrence rate) within 180 days.

Results

Of the 708 included patients, 185 (26.1%) were hyponatremic on admission. Of these, 28 (15.1%) were still hyponatremic at discharge. 34 (4.8%) patients developed hyponatremia during hospitalization despite being normonatremic on admission. Patients with hyponatremia at discharge had a higher rate of pneumonia recurrence as compared to normonatremic patients (OR 2.68; 95%-CI 1.09-6.95; p=0.037). Among patients with hyponatremia at discharge, patients who were already hyponatremic on admission showed the strongest association with increased recurrence rate (OR 4.01; 95%-CI 1.08-12.64; p=0.022). In contrast, recurrence rate was not affected in patients who were hyponatremic on admission but had normalized serum sodium levels at discharge (p=0.73).

Conclusion

Mild to moderate hyponatremia at discharge is associated with an increased risk of recurrence in hospitalized patients with pneumonia. This association is particularly strong for patients who are hyponatremic both on admission and at discharge, emphasizing the importance of hyponatremia correction during hospitalization.

Introduction

Hyponatremia, defined as a serum sodium < 135 mmol/l, is a common electrolyte disorder with a reported incidence of up to 30% in hospitalized patients[1,55,57]. Profound, symptomatic hyponatremia with a serum sodium < 125 mmol/l is less common, with a reported incidence of 1-3%, but bears the risk of life-threatening complications such as seizure, coma or death [55,57,58]. Profound hyponatremia is more likely to be promptly corrected by physicians, whereas mild to moderate hyponatremia in asymptomatic patient often remains unaddressed[1,55,59]. In patients with pneumonia, hyponatremia is particularly common, with a described incidence of up to 30%[60–64].

The underlying mechanism of hyponatremia in patients with pneumonia is poorly understood, but it is hypothesized that the physiological ADH drive during disease related stress[65,66], decreased renal water clearance, and concomitant saliuretic therapy in polymorbid, elderly patients play a major role[63,64,67].

Hyponatremia at admission is associated with a worse patients' outcome, i.e. mortality and morbidity[68–70], independent of the underlying disease. In pneumonia, hyponatremia on hospital admission was shown to be an independent risk factor for in-hospital mortality, long hospitalization and increased morbidity[59,71,72]. In line with these findings, hyponatremia on/at admission was associated with an increased mortality of up to 5 years after hospitalization with pneumonia[30].

In contrast to admission hyponatremia, no study so far has investigated the impact of discharge hyponatremia on patients' outcome in patients with pneumonia.

The aim of this study was therefore to investigate the impact of hyponatremia at discharge and the impact of hyponatremia correction on clinical outcome in hospitalized patients with pneumonia.

Material and Methods

Study design and participants

This is a preplanned secondary analysis of a multicenter, randomized, double-blind, placebo-controlled trial. Full details of the study rationale, design and statistical analyses can be found elsewhere [73]. In brief, eligible patients with CAP were treated with 50 mg prednisone or placebo for 7 days in addition to standard therapy. For the current analysis, we included all adult patients hospitalized with CAP, available serum sodium levels and treated per protocol. Patients with hypernatremia at discharge (serum sodium levels > 145 mmol/L) were excluded.

The trial was approved by the responsible ethical committees of the participating sites. Written informed consent has been obtained from all participants after full explanation of the purpose and nature of all procedures used. The trial was registered on clintrials.gov (NCT00973154) and conducted according to the declaration of Helsinki and Good Clinical Practice Guidelines.

Study procedure and outcomes

Data about baseline characteristics (e.g., age, sex, body mass index (BMI), comorbidities, Pneumonia Severity Index (PSI), vital signs) [74] were collected at study inclusion. Serum sodium measurements were routinely performed at the central laboratories of the respective hospitals on admission, at day 3, 5, 7 and at discharge, or depending on the length of hospital stay. All centres involved in the study have a standardized operating procedure (SOP) for treatment of hyponatremia according to etiology. This SOP was realized according to the guidelines published in the *European Journal of Endocrinology* in 2014 [58]. Further variables including length of hospital stay and days with symptoms of pneumonia were assessed at discharge. Patients' outcomes such as mortality, rehospitalization and recurrence rate of pneumonia were assessed 180 days after index hospitalization by telephone interview. Recurrence of pneumonia was defined as microbiological, clinical and/or radiological recurrence within 180 days after index hospitalization, both if treated ambulatory or needing a rehospitalization. Information about microbiological data included the presence of a viral, bacterial or combined

viral and bacterial infection. Microorganism were classified as pneumococcus vs no pneumococcus pneumoniae for bacterial infections and flue vs no flue in viral infection.

For this analysis, patients were divided into two groups according to serum sodium levels: hyponatremia defined as a serum sodium level < 135 mmol/l and normonatremia defined as a serum sodium level between 135 and 145 mmol/l. Patients with hyponatremia were subclassified according to severity into mild (serum sodium level between 130-134 mmol/l), moderate (serum sodium level between 125-129 mmol/l) and profound (serum sodium levels < 125 mmol/l) hyponatremia.

We performed analyses of the following subgroups: patients with hyponatremia on admission compared to patients with normonatremia on admission; patients with hyponatremia at discharge compared to patients with normonatremia at discharge. Among patients with hyponatremia on admission, we performed further subgroup analyses, distinguishing patients with persistent hyponatremia (hyponatremia both on admission and at discharge) from patients with corrected hyponatremia (hyponatremia on admission and normonatremia at discharge).

Statistical analysis

Results are shown as mean $\pm$ standard deviation (SD) or number (n) and percentage (%) unless stated otherwise. Two group comparison of continuous baseline variables were analyzed using the Wilcoxon rank sum test and of categorical baseline variables using the Chi-Square test. Count data were analyzed using a Poisson regression model.

To assess the impact of serum sodium levels on death, rehospitalization and recurrence rate we built both univariate and multivariable models for each of the outcomes. As 50% of our sample received prednisone, we included treatment arm in the multivariable model. Further known variables influencing sodium levels such as sex, age, BMI, initial PSI class and the presence of comorbidities were included in the multivariable model. As comorbidities, we had data about presence of heart failure, renal failure, COPD, active neoplastic disease and concomitant infections. We included the independent variables in a step-wise way.

Both univariate and multivariable models were applied for the comparisons of different groups (i.e. hyponatremia on admission versus normonatremia on admission; hyponatremia at discharge versus normonatremia at discharge; persistent hyponatremia vs normonatremia at discharge; corrected hyponatremia vs persistent normonatremia). Scatter plot, bar plot and Forest plot were used for graphical analysis.

Data were analysed using R statistical software[75]. A two-sided p-value of < 0.05 was considered to be statistically significant.

Results

Baseline characteristics

Data of 725 patients were available from the initial study[73]. Seventeen hypernatremic patients were excluded from the analysis. Out of the 708 included patients, 266 (37.5%) were female. The mean age was 69.2 ± 17.2 years and BMI 26.7 ± 6.4 kg/m^2. The mean length of hospitalization was 9.3 ± 17.8 days; mean days with symptoms of pneumonia was 5.8 ± 9.4. Baseline characteristics for the whole group as well as according to serum sodium levels at discharge are summarized in Table 1.

Table 1 – Patients' characteristics, normo- versus hyponatremia at discharge

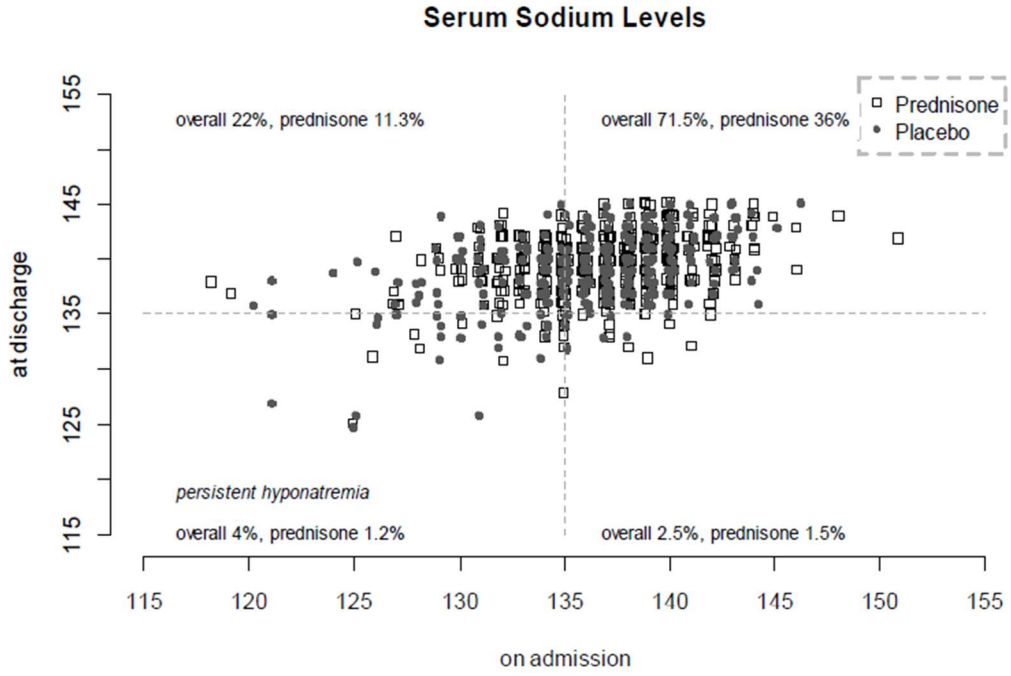
	All Participants	Hyponatremia at Discharge	Normonatremia at Discharge	p
Participants n (Prednisone treatment %)	708 (49.4)	45 (44.4)	663 (50.4)	0.56
Female sex (%)	266 (37.5)	19 (42.2)	247 (37.2)	0.64
Age (years) ± SD	69.4 ± 17.3	73.5 ± 14.5	68.9 ± 17.4	0.08
BMI (kg/m^2) ± SD	26.6 ± 6.4	25.5 ± 5.2	26.7 ± 6.5	0.17
Temperature (°C) ± SD	37.6 ± 0.9	37.6 ± 0.9	37.6 ± 0.9	1
Systolic Blood Pressure on admission (mmHg) ± SD	125.9 ± 20.9	125.0 ± 20.5	126.1 ± 21.0	0.96
Heart rate on admission (beats per minute) ± SD	84.9 ± 17.3	82.1 ± 16.7	85.0 ± 17.3	0.34
Respiratory Rate on admission (breaths per minute) ± SD	21.1 ± 5.2	21.5 ± 5.3	21.1 ± 5.1	0.65
Length of Stay (days) ± SD	9.33 ± 17.8	9.42 ± 6.3	9.33 ± 18.3	0.93
Days with symptoms ± SD	5.82 ± 9.4	3.88 ± 2.6	5.95 ± 9.6	0.09
Renal Insufficiency, n (%)	215 (30.4)	13 (28.9)	202 (30.5)	0.92
Neoplastic Disease, n (%)	47 (6.6)	4 (8.9)	43 (6.5)	0.75
Co-infections, n (%)	79 (11.1)	3 (6.7)	76 (11.5)	0.44
Confusion, n (%)	46 (6.5)	4 (8.9)	42 (6.3)	0.72
COPD, n (%)	117 (16.5)	10 (22.2)	107 (16.1)	0.41
Heart Failure, n (%)	124 (17.5)	6 (13.3)	118 (17.8)	0.57

Two group comparison of continuous variables were analyzed using the Wilcoxon rank sum test, of categorical variables using a Chi-Square test and of count data using a logistic regression model. SD= standard deviation, BMI= body mass index, COPD= chronic obstructive pulmonary disease

Patients with hyponatremia on admission and with persistent hyponatremia showed a significant lower BMI (p= 0.002 and p= 0.04, respectively).

Altogether, 185 (26.1%) patients were hyponatremic on admission. Of these patients, 140 (76.7%) had mild hyponatremia, 37 (20%) had moderate hyponatremia and 8 (4.3%) had profound hyponatremia. In 157 (84.9%) hyponatremic patients on admission the serum sodium level normalized during hospitalization, whereas the remaining 28 (15.1%) patients were still hyponatremic at discharge. Thirty-four (4.8%) patients developed hyponatremia during hospitalization. Of these, 17 (50%) patients were still hyponatremic at discharge. *Figure 1a* displays the distribution of patients according to serum sodium level on admission and at discharge stratified for randomization group.

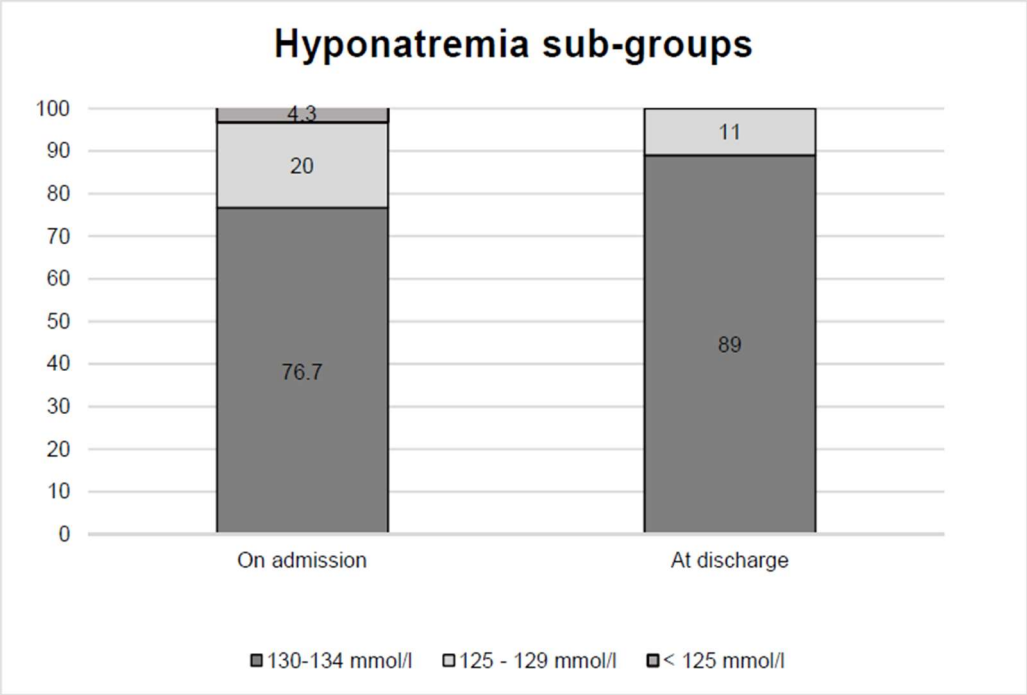
Figure 1a – Patients and Serum Sodium Levels



Distribution of patients getting prednisone or placebo according to serum sodium level on admission and at discharge

Figure 1b shows the distribution of hyponatremia subgroups on admission and at discharge.

Figure 1 b – Hyponatremia sub-groups on admission and at discharge



Data are presented as percentage of patients. The majority of patients both on admission and at discharge had a mild hyponatremia. No patient had a serum sodium level < 125 mmol/l at discharge.

Among patients with hyponatremia at discharge (n= 45, 6.3%), 40 (89%) had mild hyponatremia and 5 (11%) had moderate hyponatremia. No patient had profound hyponatremia (Figure 1b). In the prednisone group there were slightly more normonatremic patients at discharge as compared to the placebo group (334 vs 329, p= 0.5). A similar distribution was found on admission (p= 0.6). Patients in the placebo group showed a trend towards persistent hyponatremia as compared to prednisone group (9 vs 19, p= 0.07), whereas patients with persistent normonatremia were equally distributed in both groups (254 vs 252 patients, p = 1). The overall recurrence rate of pneumonia was 5.6% (n= 40), the overall mortality rate 7.2% (n= 51) and the overall rehospitalization rate was 17.1% (n= 121). There

was no statistically significant difference in death, (p= 0.4) rehospitalization (p= 0.2) and recurrence rate (p 0.6) between patients treated with or without prednisone. This was confirmed in our study including 708 patients with serum sodium < 146 mmol/l at discharge (p= 0.4 for outcome *Death*, p= 0.2 for outcome *Rehospitalization*, p= 0.6 for outcome *Recurrence* rate of pneumonia when performing a Fisher exact test for prednisone and placebo group).

Data about microbiological findings were available for 30.5% (n= 216) of the patients. We did not find any association between a positive microbiological finding (either bacterial or viral) and admission hyponatremia (p= 0.5), whereas we found an association between a positive microbiological finding and discharge hyponatremia (p= 0.009).

Hyponatremia on admission and outcome of pneumonia

The length of hospitalization was similar in both hyponatremic on admission and normonatremic patients (9.64 days vs 9.23 days, p= 0.79). However, admission hyponatremia was associated with an increased number of days with symptoms of pneumonia (6.19 days vs 5.68 days, p= 0.02).

Hyponatremia of any grade on admission was not associated with an increased recurrence rate of pneumonia within 180 days (p= 0.63, data not shown). The subgroup analysis of patients with moderate and profound hyponatremia on admission showed an association with an increased recurrence rate of pneumonia (OR 2.84, 95%-CI 1.02-6.75, p= 0.027) (Table 2a). This association remained statistically significant when adjusted for age, sex, BMI, prednisone treatment and comorbidities (adjusted OR 2.89, 95%-CI 1.02-7.14, p= 0.029) (Table 2a). No association was found between hyponatremia on admission and mortality (p= 0.60 for patients with hyponatremia of any grade; p= 0.89 for patients with a serum sodium < 130 mmol/l) nor rehospitalization rate (p= 0.61 and p= 0.56, respectively), both in the univariate and multivariable analysis (Table 2a).

Table 2a - Moderate and profound hyponatremia versus normonatremia on admission on patient's outcomes.

Hyponatremia on Admission < 130 mmol/l and Outcomes with and without confounders							
	Univariate analysis independent variable: serum sodium < 130 mmol/l				Multivariable analysis independent variables: serum sodium < 130 mmol/l, age sex, BMI, prednisone and comorbidities		
Outcomes	**OR**	**95% CI**	**p**		**OR**	**95% CI**	**p**
Recurrence	**2.90**	**1.04 - 6.92**	**0.02**		**2.89**	**1.02- 7.14**	**0.02**
Rehospitalization	1.25	0.55 - 2.58	0.56		1.33	0.57 – 2.82	0.47
Death	0.92	0.22 – 2.65	0.89		0.98	0.22 – 3.12	0.98

Odds ratios and adjusted odds ratio for age, sex, BMI, prednisone treatment and comorbidities using a logistic regression model. OR = odds ratio, 95%-CI = 95%-confidence interval, BMI = body mass index

Hyponatremia at discharge and outcome of pneumonia

The length of hospitalization was similar in both hyponatremic at discharge and normonatremic patients (9.42 days vs 9.33 days, p= 0.97). The number of days with symptoms tended to be higher in patients with hyponatremia at discharge (3.88 vs 5.97, p= 0.09) (Table 1).

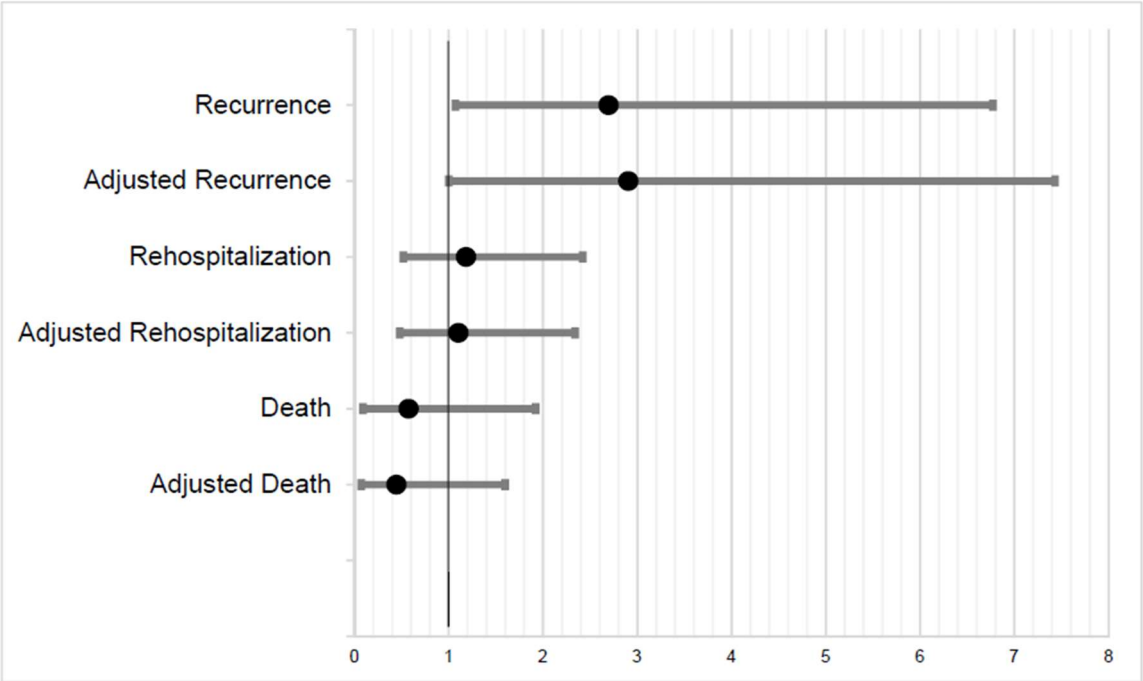
Patients with hyponatremia of any grade at discharge had a higher recurrence rate of pneumonia within 180 days compared to normonatremic patients at discharge (OR 2.68; 95%-CI 1.09-6.95; p= 0.037) (Table 2b and Figure 2). This remained statistically significant after adjusting for age, sex, BMI, PSI-class, prednisone treatment and comorbidities (OR 2.90, 95%-CI 1.00-7.43, p= 0.035) (Table 2b and Figure 2).

Table 2b - Hyponatremia of any grade at discharge versus normomatremia at discharge and patients' outcomes.

Hyponatremia at Discharge and Outcomes							
Outcomes	**OR**	**95% CI**	**p**	**Adjusted for Age, Sex, BMI, PSI, Prednisone and Comorbidities**	**OR**	**95% CI**	**p**
Recurrence	**2.90**	**1.04 - 6.92**	**0.037**		**2.90**	**1.00 - 7.43**	**0.035**
Rehospitalization	1.18	0.52 - 2.42	0.67		1.10	0.48 - 2.34	0.80
Death	0.57	0.09 – 1.92	0.45		0.44	0.07 - 1.60	0.28
Sub-group Analyses for Pneumonia Recurrence Rate							
Persistent Hyponatremia	**OR**	**95% CI**	**p**	**Resolution of Hyponatremia**	**OR**	**95% CI**	**p**
	4.01	1.08 – 12.64	**0.02**		0.86	0.32 - 1.99	0.73

Subgroup analyses: Persistent hyponatremia versus normonatremia at discharge; resolution of hyponatremia versus normonatremia on admission and at discharge. Odds ratios and adjusted odds ratio for age, sex, BMI, PSI, prednisone treatment and comorbidities using a logistic regression model. Data of overall hyponatremia (serum sodium level < 135 mmol/l). OR = odds ratio, 95%-CI = 95%-confidence interval, BMI = body mass index

Figure 2 – Hyponatremia at discharge and outcomes within 180 days



Hyponatremia was defined as serum sodium level < 135 mmol/l. Points represent the odds ratios; error bars represent the 95% CIs. Odds ratios were calculated with and without adjustment for age, sex, BMI, PSI-class, prednisone treatment and comorbidities, by a general linear model with binomial class family.

Sub-group analyses showed the strongest association for patients who were both hyponatremic on admission and at discharge (n= 28; adjusted OR 4.01, 95%-CI 1.08-12.64, p= 0.022) independently from age, sex, BMI, prednisone treatment and comorbidities (Table 2a).

There was no association between hyponatremia at discharge and mortality (p= 0.45) nor rehospitalization (p= 0.67) within 180 days (Table 2b and Figure 2). Again, these results remained consistent when adjusted for age, sex, BMI, PSI-class, prednisone treatment and comorbidities (Table 2b and Figure 2).

Discussion

The main finding of our study is that patients with hyponatremia at discharge have a higher recurrence rate of pneumonia within the first 180 days after hospitalization as compared to patients with normal serum sodium levels at discharge. To our knowledge, this is the first study showing that hyponatremia at discharge and not only on admission is associated with a worse patient's outcome in pneumonia.

Association of admission hyponatremia with worse outcome has already been shown for patients with various diseases such as liver cirrhosis, congestive heart failure and chronic kidney disease[76–78]. Further cross-sectional studies of both hospitalized and ambulatory patients with[72] and without pneumonia indicated similar findings[2,79,80]. Similarly, a meta-analysis of 81 studies including 147,948 hospitalized participants found that mild hyponatremia on admission was associated with overall mortality[81]. Herein, we confirmed the association between hyponatremia on admission and worse patient's outcome, as patients with moderate and profound hyponatremia on admission showed an increased recurrence rate of pneumonia within 180 days, independent from age, sex, BMI, prednisone treatment and comorbidities. Of note, the importance of admission hyponatremia < 130 mmol/l as a negative predictor for patient's outcome in pneumonia is evident as it is one criteria of the Pneumonia Severity Index (PSI)-score. Importantly, whether the relation between admission hyponatremia and worse patient's outcome is pure association or causality is still unclear. To shed light on this open question, a first step would be to investigate whether correction of hyponatremia leads to an improved outcome. Such a study is currently ongoing (NCT03557957)[56].

In our study, we showed an association between hyponatremia at discharge and pneumonia recurrence rate. This association was particularly strong for patients who were hyponatremic both on admission and at discharge, suggesting that hyponatremia correction in patients with pneumonia could positively influence patient's outcome and reduce morbidity in a causal way.

Pathophysiologically, both heart and immune system could be responsible for a possible causal relationship of hyponatremia and recurrence of pneumonia. In a recent review article from Portales-Castillo, it was highlighted how adaptation to hyponatremia cause an adaptive loss of taurine[82]. This acquired taurine deficiency could lead to a cardiomyopathy with reduction of ejection fraction with consequent congestion, a condition that predisposes to pneumonia. Moreover, taurine deficiency could alter macrophage and white blood cell functions, as previously described in cats[83]. Unfortunately, our knowledge of the adaptations of the heart, macrophages, and leukocytes to hyponatremia in humans and the consequences of these adaptations is limited. In our study, we did not assess cardiac and immune function and we can therefore only speculate on the reasons for association. Of note, the association between hyponatremia at discharge and recurrence rate of pneumonia was independent of the PSI, a score that accounts for comorbidities and laboratory parameters indicating the degree of illness[74]. Moreover, the association sustained when adjusting for age, sex, BMI, prednisone treatment and comorbidities. One could argue that patients with hyponatremia are sicker and thus more vulnerable to experience disease recurrence. In pneumonia, this would mean that underlying comorbidities and disease severity cause recurrence, and that hyponatremia is just a marker for disease severity.

Our cohort is a representative collective for patients with pneumonia. The prevalence of hyponatremia in our cohort of hospitalized patients with pneumonia was in line with the literature[63]. Different reasons for the high incidence of hyponatremia in patients with pneumonia have been postulated: First, hyponatremia could be linked to the stress-related release of antidiuretic hormone (ADH) as well as to dehydration[84,85]. Second, drugs such as diuretics and psychotropics often given in elderly patients are known to induce SIADH[86,87]. Third, bacteria like Legionella pneumonia seem to trigger hyponatremia[88]. We did not assess the cause of hyponatremia and had microbiological data only about one third of the patients in our study cohort and are therefore unable to draw respective conclusions. We did find an

association between a positive microbiological finding and hyponatremia at discharge; however, we could not discriminate for specimens.

In line, our patients' population age was comparable to cohorts from the literature[85]. Furthermore, as seen in other studies, women of our collective were more likely to present with hyponatremia [89,90]. Moreover, a lower BMI was associated with hyponatremia on admission[1,72] and with persistency of hyponatremia. We speculate that a lower BMI could indicate a malnourished patient with a tendency to hypoalbuminemia. Hypoalbuminemia could worsen the fluid retention triggered by pneumonia, causing a persistence of hyponatremia. However, we did not collect data about albumin, so this remains a speculation.

Solely, the recurrence rate of pneumonia in our study was lower than the one reported in previous studies (5.6% vs 9-12%)[90]. Despite the comparable collective, we did not find an association of hyponatremia with rehospitalization nor mortality, neither for hyponatremia on admission nor at discharge. Most probably, this can be explained by the shorter observation period of 180 days only in our study as compared to the longer follow-up period of up to 5 years in other studies, and by the fact that the number of events in our study was rather low[30].

Limitations and strengths of the study

Our study has some limitations. First, as it was a secondary analysis of previously collected data, the results are hypothesis generating and the ability to draw causal links between hyponatremia and outcome are limited. Second, as only few patients had an unfavorable outcome such as recurrence rate of pneumonia, rehospitalization or mortality, the results should be interpreted with caution. Third, we have no data about the etiology of hyponatremia and only limited information about microbiological findings. Fourth, all centers involved in the study have the same standardized operating procedure for correction of hyponatremia, but we had no specific data about management of patients included in the analysis. Moreover, half of the patients were undergoing an adjunctive therapy with prednisone, which is known to effect serum sodium levels and patients' outcomes. However, separate analysis and inclusion of prednisone and placebo group in our models did not indicate a significant difference.

Strengths of our study are that data have been prospectively collected in seven different centers in Switzerland, with reliable and consistent data about patients' characteristics and serum sodium levels throughout the hospitalization. Our cohort is representative and similar to other studies investigating hyponatremia and outcomes and our findings could thus indicate the importance of hyponatremia at discharge.

Conclusion

Taken together, our findings show an association between worse patient's outcome and mild and moderate hyponatremia not only on admission, but also at discharge. This finding points towards the importance of hyponatremia correction on patient's outcome. In view of the low correction rate and the low awareness for hyponatremia during hospitalization, this has important implications for patients' daily clinical management. Ultimately, ongoing intervention studies will shed light on this important issue.

Acknowledgement

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Manuscript 2: Impact of Sodium Levels on Functional Outcomes in Patients with Stroke – A Swiss Stroke Registry Analysis

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Abstract

Context: Correction of hyponatremia might represent an additional treatment for improving stroke patients' clinical outcomes.

Objective: Admission hyponatremia is associated with worse clinical outcome in stroke patients, but whether normalization of hyponatremia improves outcome is unknown. We investigated whether normalization of hyponatremia affects patients' disability, mortality, and stroke recurrence within three months; length of hospitalization, and discharge destination.

Design: This was a registry-based analysis of data collected between January 2016 and December 2018. We linked data from Swiss Stroke Registry(SSR) with electronic patients' records for extracting sodium values.

Setting: We analyzed data of hospitalized patients treated at University Hospital of Basel.

Patients: Stroke patients whose data and informed consent were available.

Main outcome measure: Modified Rankin Scale(mRS) score at three months. The tested hypothesis was formulated after SSR data collection but before linkage with electronic patients' records.

Results: Out of 1995 patients, 144(7.2%) had hyponatremia on admission; 102(70.8%) reached normonatremia, and 42(29.2%) remained hyponatremic at discharge. An increase of initial sodium was associated with better functional outcome at three months (OR 0.94, 95%CI[0.90-0.99], for a shift to higher mRS per 1 mmol/L sodium increase). Compared to normonatremic patients, patients who remained hyponatremic at discharge had a worse functional outcome at three months (OR 2.46, 95%CI[1.20-5.03] for a shift to higher mRS). No effect was found on mortality, recurrence or length of hospitalization.

Conclusions: In hospitalized acute stroke patients, persistent hyponatremia is associated with worse functional outcome. Whether active correction of hyponatremia improves outcome remains to be determined in prospective studies.

Introduction

Stroke is one of the leading causes of mortality and morbidity worldwide, with a high social and economic impact[91]. Hyponatremia, defined as serum sodium <135 mmol/L, is frequently found on admission in patients with ischemic or hemorrhagic stroke, with a prevalence between 3.9 and 45.3%[92]. Several studies reported that hyponatremia on admission is a predictor for a poor prognosis of stroke, independent of the cause of hyponatremia[93,94]. A recent meta-analysis reported that stroke patients with hyponatremia have an increased mortality risk both short-term (RR 1.61, 95% CI [1.33-1.96]; HR 1.78 95% CI [1.19-2.75]) and long-term (RR 1.77, 95% CI [1.27-2.47]; HR 2.23, 95% CI [1.30-3.82]) follow-up[95]. Furthermore, hyponatremia on admission is associated with longer hospital stay[28], as well as higher stroke severity on the National Institutes of Health Stroke Scale (NIHSS)[22,96,97], and worse functional outcome on the modified Rankin Scale (mRS)[98,99]. Whether the normalization of hyponatremia could improve patients' outcomes after stroke, as has been described for patients with heart failure[100], remains unknown.

Therefore, this observational study aimed to investigate whether normalization of serum sodium in stroke patients with hyponatremia on admission is associated with better outcomes, including mRS, recurrent stroke rate, and mortality.

Material and Methods

We retrospectively analyzed data of patients with acute ischemic stroke or intracerebral hemorrhage (ICH) treated at University Hospital Basel, prospectively collected in the Swiss Stroke Registry (SSR), supplemented by data on serum sodium levels obtained from electronic patient records (IsMed®). This analysis was approved by the Ethics Committee of Northwestern Switzerland (EKNZ).

Swiss Stroke Registry

The SSR is a nationwide multi-center registry which has been maintained prospectively since January 2014[101] and collects a standardized dataset of all patients with acute cerebrovascular events including baseline characteristics, stroke severity assessed on the National Institute of

Health Stroke Scale (NIHSS), and a follow-up assessment after 3 months, and is compulsory for all hospitals certified as Stroke Units or Stroke Centers, in line with European Stroke Organization criteria[102]. These outcomes are assessed at the clinic or through structured telephone interviews with the patient or a proxy. They include the assessment of functional outcome on the mRS, recurrent strokes, both ischemic and hemorrhagic, and death.

Data

We analyzed data of SSR patients (i) admitted between 1st January 2016 and 31st December 2018 (ii) at the University Hospital Basel (iii) with an acute ischemic stroke or ICH, (ii) available data on 3-month outcome, and (iii) available serum sodium measurements (at least 2x, one on admission, one before discharge). Exclusion criteria were (i) missing data of 3 month follow-up, (ii) less than 2x documented serum sodium levels during the index hospitalization, (iii) hypernatremia at discharge, defined as a serum sodium level >145 mmol/L.

Serum sodium levels data were obtained from electronic patient records (IsMed®). Patients were identified using the case number, a 10 digit-number reported both in SSR and IsMed® that uniquely identifies a single hospitalization of a single patient. Serum sodium levels were extracted using the codes NAT and C-PH-NA-01, two unique codes for serum sodium levels pre-settled in the laboratory system of University of Basel, one for 2016 and 2017 (NAT) and one for 2018 (C-PH-NA-01). All sodium levels measured during the index hospitalization were extracted from electronic patient records and a randomly selected sample of it (n= 82 patients) was double-checked for consistency by an independent investigator. Data extracted from SSR and data extracted from IsMed® have been merged creating a new password-protected database in comma-separated values (csv) file. Data are safely stored by clinical trial unit of university of Basel and accessible by the investigators only after explicit request, and only as reader.

Normonatremia was defined as a serum sodium level between 135 and 145 mmol/L, hyponatremia as a serum sodium level < 135 mmol/L, as previously described[13].

For our analysis, patients were divided into four subgroups according to serum sodium levels and their evolution during hospitalization:(i) patients with hyponatremia neither on admission

nor at discharge (*normonatremia*); (ii) patients with hyponatremia both on admission and at discharge (*in-hospital persistent hyponatremia*); (iii) patients with normonatremia on admission and hyponatremia at discharge (*in-hospital acquired hyponatremia*); and (iv) patients with hyponatremia on admission and normonatremia at discharge (*in-hospital normalized hyponatremia*). Differences among the subgroups were calculated using standardized mean differences (SMDs)[103].Due to the lack of data, serum sodium levels were not corrected by glycemia levels.

Outcomes

The primary outcome was the functional outcome measured by the mRS three months after stroke. Secondary outcomes were 1) death within three months after the initial event; 2) recurrent stroke (ischemic stroke or ICH) within three months after the initial event; 3) length of hospital stay (LOS, expressed in days); and 4) discharge destination ((i)home, (ii) rehabilitation hospital, (iii) other acute care hospital, (iv) nursing home or palliative care center).

Statistical Analysis

Data were analyzed using R software[104] version 4.0.2.

We described baseline characteristics as the percentage of participants in case of categorical variables, and in median and interquartile range (IQR) for continuous variables. The analysis was performed for the whole group of participants, as well as comparing the four predefined subgroups of patients, as described above.

The primary outcome was analyzed by ordered logistic regression based on the cumulative link model (shift-analysis). The reported odds ratios refer to a one-point shift to a higher mRS score. We estimated the ordered logistic model using sodium concentration on admission and its change as continuous independent explanatory variables. We repeated the analysis using the four subgroups of patients as an independent explanatory 4-level categorical variable. For this analysis, the reference group was *normonatremia*. Moreover, we conducted a descriptive analysis of the primary outcome according to hyponatremia status on admission and at discharge (patients with hyponatremia on admission vs patients with normonatremia on

admission; and patients with hyponatremia at discharge vs patients with normonatremia at discharge), using standardize mean differences (SMD).

For the secondary outcomes, we used standard logistic regression to analyze the associations between sodium levels and mortality three months after stroke; multinomial regression models for the association with recurrence of stroke as well as for the association with discharge destination; and linear regression with the logarithm of the hospitalization days as dependent variable for the association with LOS.

We conducted the described analyses for primary and secondary outcomes both on the set of all completely observed cases and using multiple imputations, as sensitivity analysis.

In line with previous findings[105–114], variables considered as potential confounders and adjusted for in all analyses were age[105], sex[106], body-mass-index (BMI)[107,108], smoking status[109], NIHSS score on admission[110], type of stroke[111], pre-hospital mRS score, and presence of comorbidities, which included arterial hypertension, diabetes mellitus, hyperlipidemia, atrial fibrillation, coronary heart disease, and peripheral artery disease[113,114]. The analysis was carried out at the Clinical Trial Unit at the Department of Clinical Research at the University of Basel.

Results

Sample

Altogether, we analyzed data of 2`534 SSR patients. We excluded 221 patients for whom the mRS three months after the index stroke was not available; 156 cases, for whom less than two sodium measurements during their hospitalization were available in the electronic patient records; and 162 patients with hypernatremia at discharge, defined as a serum sodium level >145 mmol/L. This left us with 1`995 cases in the full analysis set. Due to missing data about pre-hospital mRS or BMI, the full set analysis of primary outcome and secondary outcomes 1), 2) and 3) was made on 952 patients, and the full set analysis of secondary outcome 4) on 919 patients who were discharged from hospital. Details on missing data according to serum sodium levels subgroups are available in *supplemental material*[115].

Baseline characteristics

Out of 1`995 patients, 1`800 (90.2%) had an ischemic stroke, and 195 (18.8%) an intracerebral hemorrhage. Male patients were 56.7% (n=1131) of the sample. The median age of the patients was 76.0 years (interquartile rage (IQR) 64.5-83.6); the median body mass index (BMI) was 25.2 kg/m^2 (IQR 23.0-27.8). Patients with hyponatremia on admission (n=144/1995, 7.2%) were older (standardized mean difference (SMD) 0.264), more often female (SMD 0.221), active smokers (SMD 0.131), had a lower BMI (SMD 0.286), and more often a history of arterial hypertension (SMD 0.205) compared to normonatremic patients. Patients with hyponatremia at discharge (n= 108/1995, 5.4%) differed in the same way for age (SMD 0.290), sex (SMD 0.221), BMI (SMD 0.249) and history of arterial hypertension (SMD 0.223), but not for smoking status (SMD 0.038). Table 1 summarizes the baseline characteristics of the whole group and the four predefined subgroups according to hyponatremia status.

Table 1 – Baseline Characteristics

	Whole Sample	Normonatremia	In-Hospital Persistent Hyponatremia	In-Hospital Acquired Hyponatremia	In-Hospital Normalized Hyponatremia	SMD
Number Of Patients, n (%)	1995 (100)	1785 (89.5)	42 (2.2)	66 (3.3)	102 (5.0)	--
Age in Years Median (IQR)	76.0 (64.5-83.6)	75.5 (64.1-83.5)	79.1 (70.6-86.5)	78.5 (68.6-82.8)	79.8 (68.3-83.6)	0.182
Sex = Male n (%)	1131 (56.7)	1035 (58.0)	21 (50.0)	29 (43.9)	46 (45.1)	0.158
BMI in kg/m^2 Median, (IQR)	25.2 (23.0-27.8)	25.3 (23.1-28.0)	24.5 (21.9-25.6)	24.0 (21.4-27.0)	24.0 (22.0-27.6)	0.226
Active Smoker n (%)	360 (18.0)	313 (17.5)	7 (16.7)	14 (21.2)	26 (25.5)	0.124
NIHSS on Admission Median (IQR)	4.0 (2.0-9.0)	4.0 (2.0-9.0)	5.5 (3.0-9.2)	5.5 (3.0-11.0)	6.0 (3.0-12.8)	0.181
Ischemic Stroke, n (%)	1800 (90.2)	1616 (90.5)	38 (90.5)	54 (81.8)	92 (90.2)	0.129
Pre-mRS n (%) 3-5	132 (6.6)	117 (9.0)	3 (9.1)	4 (10.0)	8 (10.8)	0.036
Arterial Hypertension n (%)	1557 (78.0)	1377 (77.1)	36 (85.7)	57 (86.4)	87 (85.3)	0.122
Diabetes Mellitus n (%)	449 (22.5)	391 (21.9)	8 (19.0)	20 (30.3)	30 (29.4)	0.160
Hyperlipidemia n (%)	1171 (58.7)	1060 (59.4)	21 (50.0)	41 (62.1)	49 (48.0)	0.174
Atrial Fibrillation n (%)	525 (26.3)	452 (25.3)	13 (31.0)	22 (33.3)	38 (37.3)	0.138
Coronary Heart Disease, n (%)	375 (18.8)	324 (18.2)	6 (14.3)	19 (28.8)	26 (25.5)	0.209
Peripheral Artery Disease, n (%)	114 (5.7)	95 (5.3)	3 (7.1)	6 (9.1)	10 (9.8)	0.097

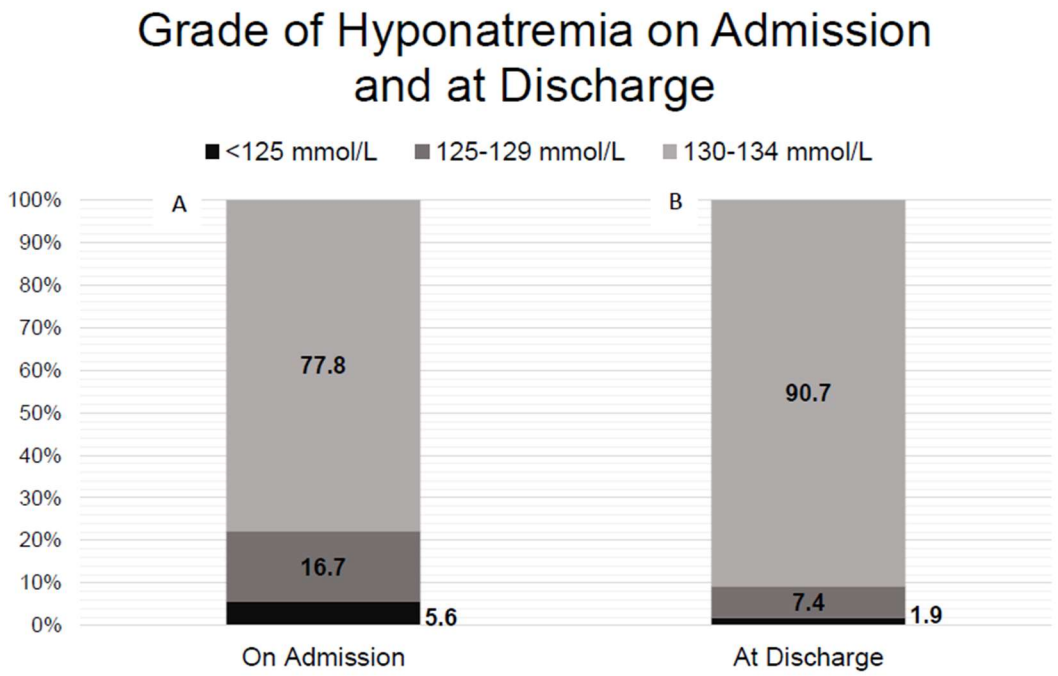
Table 1: Baseline characteristics of the whole sample of stroke patients and according to hyponatremia status. Data are expressed in median and interquartile range for continuous variables and number and percentage of patients for categorical variables.

Normonatremia= Hyponatremia neither on admission nor at discharge; In-Hospital Persistent Hyponatremia= Hyponatremia both on admission and at discharge; In-Hospital Acquired Hyponatremia = Hyponatremia at discharge, but not on admission; In-Hospital Normalized

Hyponatremia= Hyponatremia on admission, but not at discharge. SMD= standardized mean difference; IQR= interquartile range; NIHSS= National Institute of Health Stroke Scale; mRS= modified Rankin Scale

Figure 1 reports the grade of hyponatremia on admission (A) and at discharge (B).

Figure 1 – Grade of Hyponatremia on Admission and at Discharge



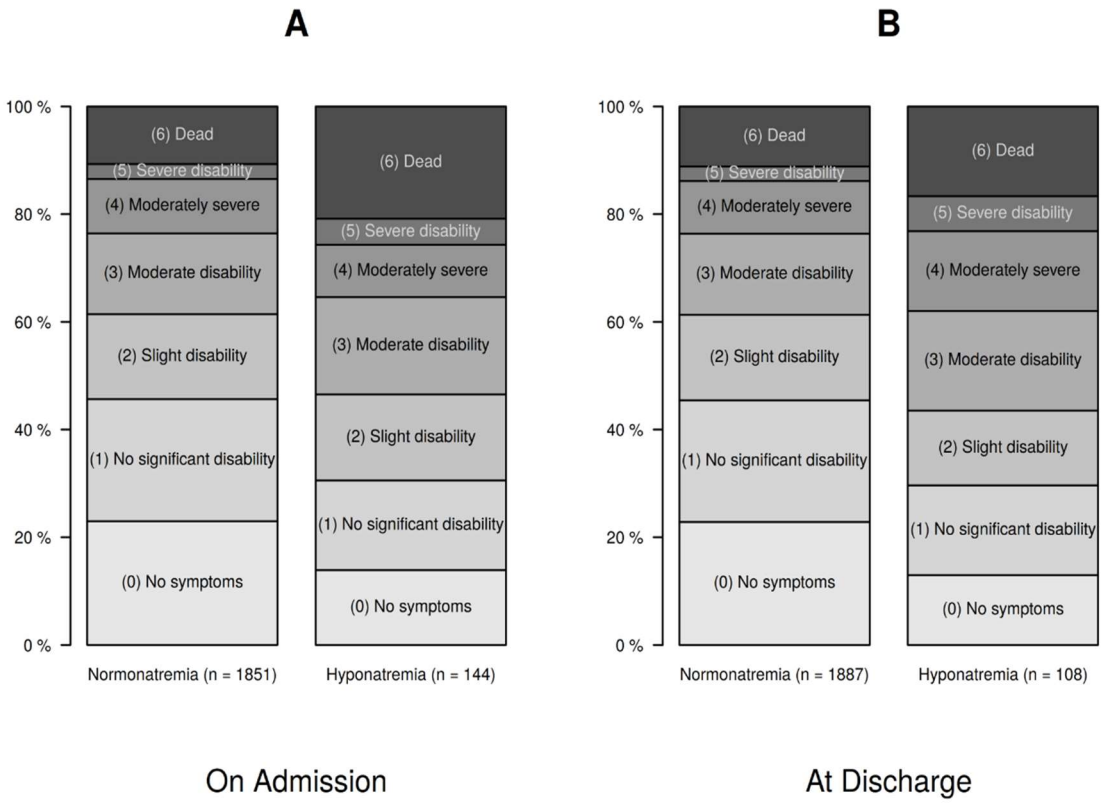
Distribution of hyponatremia severity according to

A) all patients with hyponatremia on admission (patients with in-hospital persistent hyponatremia, n=42, and patients with in-hospital normalized hyponatremia, n=102) B) all patients with hyponatremia at discharge (subgroup of patients with in-hospital persistent hyponatremia, n=42, and in-hospital acquired hyponatremia, n=66).

Hyponatremia and clinical outcomes – descriptive analysis

Patients with hyponatremia on admission (SMD 0.389) and those with hyponatremia at discharge (SMD 0.403) showed a worse functional outcome at three months compared to normonatremic patients (*Figure 2*).

Figure 2 – mRS Score at 3 Months Stratified for Normonatremia and Hyponatremia on Admission and at Discharge



mRS score at 3 months stratified by serum sodium levels on admission A and at discharge B.

Impact of admission serum sodium levels on outcomes – multivariable models

In the multivariable models of the complete case analyses, serum sodium level on admission (per mmol/L) was inversely associated with the mRS at three months (OR 0.92, 95% CI [0.88-0.96], *Table 2*), and mortality at three months (OR 0.90, 95% CI [0.84-0.97]).

Table 2 – Multivariable Analyses Results

	OR	95%CI	P VALUE
Serum Sodium on Admission (per mmol/L)	0.92	0.88-0.96	<0.001
Increase of Initial Serum Sodium (per mmol/L)	0.94	0.90-0.99	0.013
In-Hospital Persistent Hyponatremia*	2.46	1.20-5.03	0.014
In-Hospital Acquired Hyponatremia*	1.69	0.78-3.63	0.19
In-Hospital Normalized Hyponatremia*	1.30	0.75-2.25	0.35

*binomial models

Results of multivariable models with modified Rankin Scale (mRS) at three months as dependent variable. Estimation based on complete cases. The binomial models show a comparison with patients of normonatremia subgroup. All models are adjusted for age, sex, BMI, smoking status, type of stroke, National Institute of Health Stroke Scale (NIHSS) and mRS before admission, and presence of comorbidities as independent variables. The estimation for serum sodium on admission and increase of serum sodium as continuous variables did not include patients with hypernatremia at discharge. OR = Odds Ratio; CI = Confidence Interval; In-Hospital Persistent Hyponatremia= Hyponatremia both on admission and at discharge; In-Hospital Acquired Hyponatremia = Hyponatremia at discharge, but not on admission; In-Hospital Normalized Hyponatremia= Hyponatremia on admission, but not at discharge.

Moreover, serum sodium level on admission correlated with returning home as discharge destination after the index hospitalization as compared to being discharged to a rehabilitation hospital (OR 0.91, 95% CI [0.90-0.93]), to being discharged to another hospital (OR 0.88, 95% CI [0.86-0.89]), or to being discharged to a nursing home or palliative care center (OR 0.83, 95% CI [0.81-0.85]). We did not find strong evidence for an association between serum sodium levels on admission and the odds for experiencing a second event within three months (OR 0.96, 95% CI [0.86-1.07] for ischemic stroke; OR 0.90, 95% CI [0.87-0.94] for hemorrhagic stroke), or with the length of hospitalization (estimate 0.99, 95% CI [0.97-1.01]). These results were confirmed in the multiple imputation analysis (see *supplemental material*, *Tables II-V*)[115].

Impact of an increase of admission serum sodium levels on outcomes – multivariable models

Increase of admission serum sodium levels during the hospitalization within the upper limit of normality predicted a better functional outcome, being inversely associated with mRS at three months (per mmol/L: OR 0.94, 95% CI [0.90-0.99], *Table 2*). Moreover, sodium increase was associated with returning home as discharge destination after the index hospitalization as compared to being discharged to a rehabilitation hospital (OR 0.90, 95% CI [0.85-0.95]), or going to a nursing home or palliative care center (OR 0.81, 95% CI [0.73-0.91]). We did not find a strong association between increase of serum sodium and death or a second event within 3 months (death: OR 0.97, 95% CI [0.89-1.05]; recurrence: OR 0.97, 95% CI [0.85-1.10] for ischemic stroke, and OR 0.96, 95% CI [0.84-1.10] for hemorrhagic stroke), with length of hospitalization (estimate 0.98, 95% CI [0.96-1.00]), or with being discharged to another hospital (OR 0.92, 95% CI [0.85-1.00]).

Analysis by sodium group – multivariable models

Patients with hyponatremia both on admission and at discharge (*in-hospital persistent hyponatremia*) had a higher mRS at three months (OR 2.46, 95% CI [1.20-5.03], *Table* 2). Moreover, these patients had higher odds for being discharged to a nursing home or palliative care center rather than home (OR 11.12, 95% CI [2.08-59.36]). Patients experiencing a normalization of the initial hyponatremia (*in-hospital normalized hyponatremia*) showed no

clear association with a worse functional outcome at three months compared to the normonatremia subgroup (OR 1.3, 95% CI [0.75-2.25], *Table 2*). Again, the multiple imputation analysis confirmed results and trends (see *supplemental material, Tables II-V*) [115].

Discussion

Our main findings were two-fold: First, lower sodium levels on admission are associated with worse prognosis whereas increase of sodium levels during hospitalization within the upper limit of normality is associated with better patient outcomes in stroke patients. Second, in-hospital persistent hyponatremia is associated with a higher risk of functional disability and discharge destinations others than home.

Different studies showed that hyponatremia on admission in stroke patients is independently associated with increased mortality and morbidity[28,99]. Our data confirm that low serum sodium levels on admission in stroke patients are associated with worse clinical outcomes. We found an independent association with a higher level of disability three months after index stroke, and with discharge destinations other than home after the hospitalization for the index stroke. Interestingly, no study so far has investigated if normalization of the initial hyponatremia could improve the outcome of stroke patients. Here, we provide evidence that an increase of admission serum sodium levels within the upper limit of normality is associated with better outcome, (i.e., lower risk of disability three months after initial event), and higher probability of being discharged home. Specifically, we found that each mmol/L increase of initial serum sodium level was independently associated with 6% lower odds of a worse mRS at three months, and up to 1.23 times higher odds of being discharged home compared to other discharge destinations. Vice versa, patients with in-hospital persistent hyponatremia (i.e. hyponatremia both on admission and at discharge) showed a more than doubled odds for a worse mRS at three months as compared to normonatremic patients.

In the past years, hyponatremia was reported to be associated with worse prognosis in several other diseases, such as heart failure[116], myocardial infarction[117], liver cirrhosis[68], pneumonia[72,118], pulmonary embolism[119], renal insufficiency[120] and general outcome of

patients on the intensive care unit[121], with increased mortality and morbidity in patients with hyponatremia as compared to normonatremic ones.

Interestingly, in our cohort, in contrast to a higher risk for disability, neither in-hospital persistent nor in-hospital acquired hyponatremia were strongly associated with an increased mortality rate at three months. This might simply reflect lack of power, as both the prevalence of hyponatremia in our sample (7.2% vs a previously described prevalence up to 45.3%)[92], as well as 3-month mortality (11.4% vs previously described 20% respectively up to 25% for hemorrhagic stroke[122]) were relatively low. Nevertheless, our data show that in hyponatremic patients increase of sodium levels within the upper limit of normality is associated with a better functional outcome, and persisting hyponatremia is associated with a worse outcome. However, our data does not allow to make a statement if active treatment or spontaneous remission led to the increase in serum sodium levels. To resolve this question, a prospective study comparing active treatment versus no treatment in hospitalized patients with hyponatremia would be needed.

The reason by which persistency of admission hyponatremia could worsen clinical outcomes in stroke patients remains unclear. Animal models have shown that chronic hyponatremia leads to a loss of intracellular organic molecules such as taurine and glutamate as a compensatory mechanism[123]. The accumulation of glutamate in extracellular space as by mutations in glutamate transporter causes motor discoordination and alteration of long-term potentiation in the hippocampus in mice and rats[124]. This extracellular accumulation could lead to postsynaptic inhibitory effects and motor discoordination also in humans[4,125]. In agreement with this hypothesis, clinical studies documented cognitive impairment and gait abnormalities in patients with chronic hyponatremia[126,127], underlining the potential role of a low serum sodium level in impairing neurocognitive and motor performance. Moreover, a recent study from Suarez et al. showed at least a trend to better neurocognitive and motor performances following increase of serum sodium levels within the upper limit in hospitalized, hyponatremic patients with euvolemic hyponatremia[128]. However, since we did not measure taurine and glutamate levels in our study, this remains speculative.

Our study has several limitations. First, the observational design limits the generalizability of our study. Second, information about the etiology of hyponatremia was lacking. However, previous analyses have shown an association between hyponatremia and worse outcome in stroke patients, independent from hyponatremia origin[95]. Third, we did not have data about glycemia and could therefore not correct the serum sodium levels for glucose levels. However, the distribution of patients with diabetes mellitus was similar in the groups of patients with normo- and hyponatremia. Moreover, we added presence of diabetes mellitus as confounder in the analysis, and could therefore show an independent effect of serum sodium levels on the outcomes. Fourth, due to the observational nature of our study, we could not clarify whether normalization of serum sodium was spontaneous or the result of an active correction. Moreover, we did not have enough data to directly compare patients with persistent hyponatremia and patients with normalization of initial hyponatremia. Altogether, our data suggest that persistency of hyponatremia is associated with worse patients' outcome, but we cannot clarify whether this association means causality or simply defines hyponatremia as a marker for a worse outcome. Here, randomized controlled trials are required to determine whether an active correction of hyponatremia versus no correction could modify patients' outcome.

In conclusion, our study shows an association of hyponatremia not only on admission, but also at discharge, with a worse functional outcome in patients with an acute ischemic or hemorrhagic stroke. No clear association with worse functional outcome was found if initial hyponatremia normalized during hospital stay. An increase in serum sodium during hospitalization within the upper limit of normality was associated with improved functional outcome. Intervention studies are needed to answer the question if active correction of serum sodium improves clinical outcome after stroke.

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Data availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Manuscript 3: Effect of Hyponatremia Normalization on Osteoblast Function in Patients with SIAD

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Abstract

Objective: Hyponatremia is associated with increased risk of bone fragility and fractures. Many studies suggest that hyponatremia stimulates osteoclast activation, whereas other studies rather reveal a possible role of acute hyponatremia in impairing osteoblast function. We aimed to assess whether and how correction of hyponatremia in hospitalized patients with syndrome of inappropriate antidiuresis (SIAD) has an impact on bone metabolism.

Design and Methods: This was a pre-defined secondary analysis of 88 hospitalized patients with SIAD undergoing a randomized treatment with SGLT-2 inhibitors or placebo for 4 days. Biochemical markers of bone resorption (CTX) and bone formation (PINP) were collected in serum at baseline and after the intervention (day 5). Bone formation index (defined as PINP/CTX) and its difference between day 5 and baseline were calculated. Patients with steroid therapy (n=6), fractures (n=10), or whose data were missing (n=4) were excluded from the analysis.

Results: Out of 68 patients, 27(39.7%) were normonatremic at day 5. These patients showed an increase in serum PINP (p=0.04), whereas persistent hyponatremic patients did not (p=0.38), with a relevant difference between these two subgroups (p=0.005). Serum CTX increased similarly in the two groups (p=0.43). This produced a 47.9 points higher PINP/CTX difference between discharge and admission in normonatremic patients (95%CI 17.0-78.7, p=0.003) compared to patients with persistent hyponatremia, independent of age, sex, BMI, smoking habits, randomization arm, and baseline cortisol levels.

Conclusions: Our predefined post-hoc analysis shows that correction of hyponatremia in hospitalized patients with SIAD might have a positive impact on osteoblast function.

Introduction

Hyponatremia, the most frequent electrolyte disorder in hospitalized patients[1,80], has been associated with bone loss, osteoporosis, fragility and bone fractures[33,129,130]. Many retrospective studies in humans described a sex-independent association between hyponatremia and fractures[131,132]. These findings have been confirmed in a big database analysis, showing increased odds for osteoporosis and fragility fractures for subjects with acute and chronic hyponatremia compared to normonatremic subjects[36]. The fracture risk exceeded the osteoporosis risk in all categories[36]. Verbalis et al showed in animal models that induction of hyponatremia directly leads to changes in bone structure, suggesting a causal relationship of hyponatremia and bone loss[133]. Supporting these findings, a case report described recovery from osteoporosis in a young patient with hyponatremia due to tumor-induced syndrome of inappropriate antidiuresis (SIAD) after excision of the tumor[32].

The mechanism underlying the association between hyponatremia and bone loss has been studied in animal models and cell cultures. Data from a rat model of SIAD showed a reduction of 30% of both cortical and trabecular bone mass in hyponatremic rats due to a 5-fold increase in osteoclast number compared to samples from normonatremic controls[133] . Similarly, in another rat model it was confirmed that hyponatremia induces a mobilization of bone sodium stores and therefore an increase in bone catabolism[134]. Hyponatremic rats were shown to have an increased loss in lean mass as compared to normonatremic controls, again increasing the risk for osteoporosis and fragility fractures.

Cell culture studies[40,135] showed that osteoclasts grown in hyponatremic medium are more active than the ones grown in normonatremic medium. These studies also showed that osteoblasts grown in hyponatremic medium express more cytokine genes promoting the attachment of osteoclast precursors to the bone, and therefore bone resorption, as compared to osteoblasts grown in normonatremic medium. Moreover, hyponatremia induced adipocyte instead of osteoblast differentiation of mesenchymal stromal cells.

Despite this increasing knowledge of an association between hyponatremia and altered bone metabolism, studies investigating the mechanism underlying this association in humans are rare, as they would imply invasive methods such as multiple bone-biopsies. Recently, an analysis of hospitalized patients with acute hyponatremia revealed the importance of bone markers for a non-invasive bone metabolism assessment, and showed that acute mild hyponatremia is associated with a reduction of bone formation[53]. So far, only an exploratory secondary analysis comparing the effect of tolvaptan versus placebo investigated the effect of an intervention for correction of hyponatremia on bone markers in humans, showing a possible effect both on osteoblast and osteoclast activation[54]. However, this was an exploratory efficacy end point analysis on a limited number of patients.

The goal of our study was therefore to assess whether and how correction of hyponatremia in hospitalized patients with SIAD has an impact on bone metabolism assessed by measurement of plasma bone turnover markers.

Material and Methods

Patient sample

This was a pre-defined secondary analysis of 88 hospitalized patients with SIAD induced hyponatremia, undergoing treatment with the SGLT-2 inhibitor empagliflozin or placebo in addition to standard fluid restriction of < 1000 ml/24 h for 4 days in a prospective, double-blind, randomized trial conducted at the University Hospital of Basel between September 2016 and January 2019. The local ethics committee *Ethikkommission Nordwest- und Zentralschweiz* (EKNZ 2015–00131) approved the study protocol and the trial was registered at ClinicalTrials.gov (NCT02874807). Written informed consent was obtained from all patients. The original study was published elsewhere[136]. Patients with SIAD-induced hyponatremia < 130 mmol/L and 18 years of age or older were eligible. SIAD was defined by clinical assessment of euvolemia, a plasma osmolality < 275 mmol/kg, urine osmolality > 100 mmol/kg, urine sodium > 30 mmol/l and absence of hypocortisolismus or hypothyroidism. Symptomatic hyponatremia requiring a treatment with 3% NaCl solution, renal impairment, hepatic

impairment, systolic blood pressure < 90 mm Hg, diabetes mellitus type 1, participation in another study, pregnancy, breastfeeding, end of life care, or current treatment with SGLT2 inhibitors, lithium chloride, or urea were exclusion criteria. We also excluded patients with a contraindication for lowering blood pressure (e.g., subarachnoid bleeding), severe immunosuppression (leukocytes < 2 g/L), or peripheral arteriovascular disease stage 3 or 4.

Laboratory Analysis

Serum sodium levels were measured daily, whereas biochemical markers of bone resorption (C-terminal telopeptide, CTX) and bone formation (N-terminal propeptide of type I collagen, PINP) were collected in serum at baseline and at day 5 corrected for pre-analytical bias (blood sampling in the morning between 8 and 10 a.m., before breakfast). CTX and PINP were analyzed in one block and measured in serum with electrochemiluminescence immunoassays (ECLIA) on the automated analyzer cobas® e411 (Roche Diagnostics, Rotkreuz/Switzerland). The intra- and interassay variation was 2.0-8.4% for CTX and 1.2-3.3% for PINP, respectively. Bone formation index, defined as PINP/CTX, was calculated. The difference between bone formation index at day 5 and at baseline was defined as bone metabolism marker. Copeptin, a well-known parameter for indirect measurement of AVP[137,138], was also measured at baseline and at day 5.

Statistical analysis

This was a full set analysis. The primary endpoint was the difference in bone metabolism between patients with correction of hyponatremia versus patients with persistent hyponatremia. Secondary endpoints were the difference in PINP, CTX, and PINP/CTX levels from study begin to day 5 in patients with hyponatremia correction versus hyponatremia persistency.

Baseline characteristics are shown as % of participants in case of dichotomic variables, as median and interquartile range (IQR) in case of continuous, not normally distributed variables and in mean +/- standard deviation (SD) in case of continuous, normally distributed variables. *Mann-Whitney test* was implemented to compare two groups of continuous variables, whereas

a *chi-square test* or a *Fisher exact test* was used to compare categorical variables. Boxplots were used to display differences between the subgroups of patients with persistency of baseline hyponatremia versus patients with correction of baseline hyponatremia.

As marker for bone metabolism, we investigated the bone formation index (ie, PINP/CTX ratio) and its dynamic between baseline and day 5 (Difference between PINP/CTX ratio at day 5 and PINP/CTX ratio at baseline). After a descriptive between group analysis using a *Mann-Whitney test*, we computed a linear regression model with the difference between PINP/CTX ratio at day 5 and PINP/CTX ratio at baseline as dependent variable, and normonatremia at day 5 as independent variable in order to adjust for confounders. For this purpose, age, sex, BMI, smoking status, baseline cortisol level, intervention arm (drinking restriction and placebo vs drinking restriction and SGLT2-inhibitor therapy), and the use of thiazide or loops diuretics were implemented as confounders as additional independent variables. Parsimony principle was implemented in choosing the confounders for the multivariable model[139]. We excluded multicollinearity by calculating the variance inflation factor (VIF)[140]. Data analysis was carried out using R software[104].

Results

For the purpose of this study, patients with steroid therapy (n= 6), fractures or bone metastasis (n= 10), and/or whose blood samples were missing (n= 4) were excluded, leaving us with a full set of 68 patients (34 receiving empagliflozin and 34 receiving placebo) out of the initial 88 patients. Baseline characteristics of the whole cohort as well as for the subgroups of patients according to sodium status (i.e. hyponatremia or normonatremia at day 5) are displayed in *Table 1*.

Table 1 – Baseline Characteristics

	Whole sample n= 68	Hyponatremia at day 5 n= 41	Normonatremia at day 5 n= 27	p (Hyponatremia vs Normonatremia)
Age in years Mean +/- SD	77.0 (13.76)	77.0 (15.4)	76.0 (10.9)	0.62
Sex female n (%)	40 (58.8)	26 (63.4)	14 (51.9)	0.49
BMI kg/m2 Median (IQR)	22.6 (20.3-26.6)	21.6 (19.6-24.1)	24.2 (21.8-27.1)	0.09
Smoking Status yes n (%)	14 (20.6)	9 (21.9)	5 (18.5)	0.97
Intervention with SGLT-2 inhibition, n (%)	34 (50)	17 (41.5)	17 (63.0)	0.19
Baseline Serum Cortisol in mmol/l, Median (IQR)	467 (402-558)	462 (413-533)	471 (381-586)	0.96
Baseline Serum Sodium in mmol/l, Median (IQR)	125 (122-127)	126 (123-127)	125 (122-127)	0.43
Baseline Serum Calcium in mmol/l, Median (IQR)	2.38 (2.32-2.46)	2.37 (2.31-2.42)	2.39 (2.34-2.48)	0.27
Thiazide Diuretics, n (%)	24 (58.5)	10 (24.4)	14 (51.8)	0.04
Loop Diuretics, n (%)	9 (13.2)	3 (7.3)	6 (22.2)	0.14*
Baseline PINP/CTX ratio, Median (IQR)	87.5 (66.2-123.7)	96.6 (71.5-130.2)	81.8 (63.7-105.7)	0.27

** Fisher exact test*

Baseline characteristics for the whole group as well as according to subgroups obtained considering serum sodium level status at day 5. P values are calculated with *Mann-Whitney test* for continuous variables and with *Chi-square test* or *Fisher exact test* for categorical variables, depending on the number of cases. SD= standard deviation; BMI= body mass index; IQR= interquartile range; SGLT-2 = sodium-glucose co-transporter 2; PINP= N-terminal propeptide of type I collagen; CTX= C-terminal telopeptide.

Out of the initial 68 hyponatremic patients, 39.7% (n= 27/68) were normonatremic at the end the intervention. Patients were on average 77 +/- 13.8 years old, with a BMI of 22.6 kg/m^2 (IQR 20.3-26.6 kg/m^2). 59% (n= 40/68) were females, and 21% (n= 14/68) were smokers. Patients with a persistency of hyponatremia and patients who became normonatremic showed no relevant differences in baseline characteristics, except for the use of thiazide diuretics (Table 1). Of note, serum calcium levels were very similar in the two subgroups (p= 0.27). Moreover, baseline copeptin levels were similar in the two subgroups (p= 0.63).

Causes of SIAD were lung disease (n= 4/68, 5.9%), central nervous system disease (n=7/68, 10.3%), drug induced (diuretics, antipsychotic, or antiepileptic drugs, n=22/68, 32.3%), psychiatric disease (n=18/68, 26.5%), and others, defined as pain, stress or unknown (n=17/68, 25.0%). There was no difference in causes of SIAD in the two subgroups of patients with and without correction of hyponatremia at day 5 (p> 0.1).

Baseline CTX and PINP were very similar between the two subgroups of patients with and without later correction of hyponatremia at day 5 (median (IQR) 0.40 (0.23-0.49) vs 0.39 (0.30-0.57) ng/ml, p= 0.51 for CTX; and 33.5 (23.4-45.0) vs 35.2 (23.6-39.6) ng/ml, p= 0.84 for PINP).

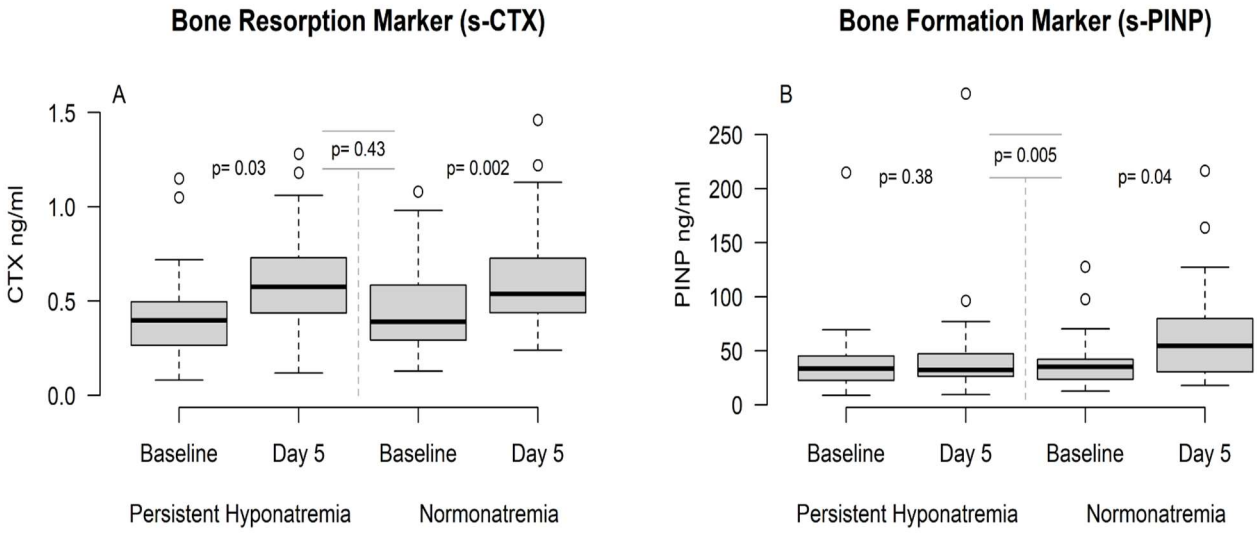
The bone formation index PINP/CTX at baseline was also similar between the two subgroups (96.6 (71.5-130.2) vs 81.8 (63.7-105.7), p= 0.27).

Influence of correction of hyponatremia on bone markers

CTX as marker for bone resorption increased during the hospitalization both in patients with (p=0.03) and without (p=0.002) correction of initial hyponatremia, without a significant difference between the two subgroups (p= 0.43), as displayed in *Figure 1a*.

PINP as marker for bone formation increased in patients who reached normonatremia (p= 0.04), whereas it remained stable in patients with persistent hyponatremia (p= 0.38), with a relevant difference between the two subgroups (p= 0.005), as displayed in *Figure 1b*.

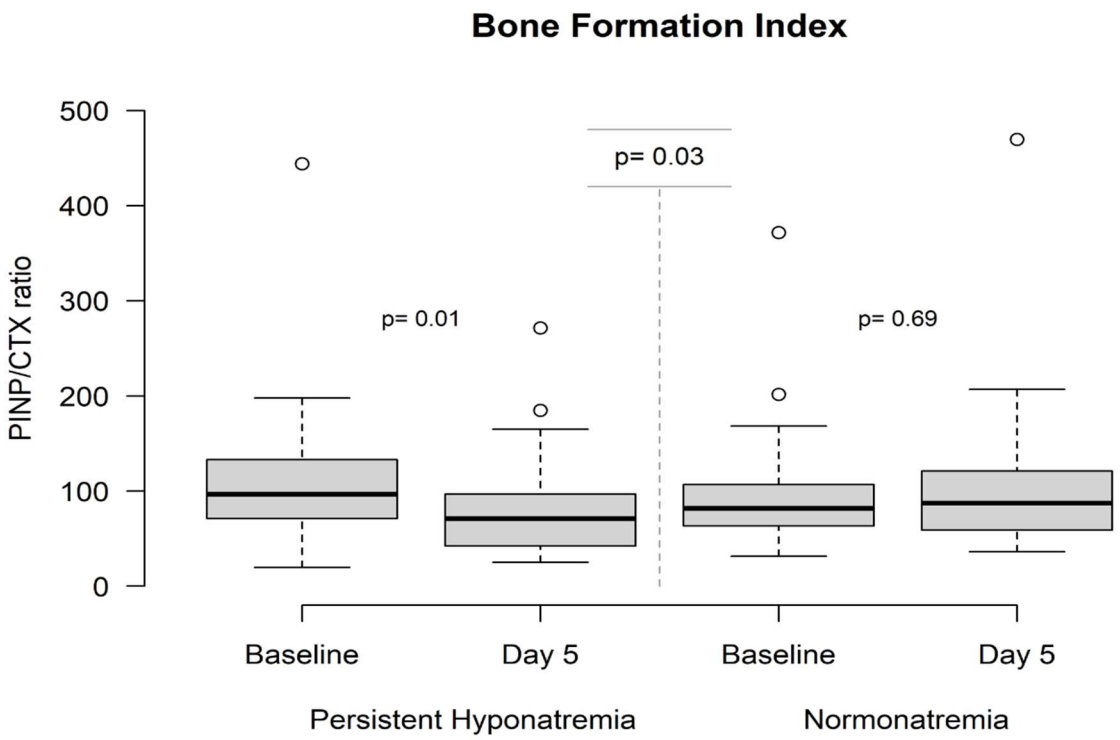
Figure 1 – Bone Resorption and Bone Formation Markers



Boxplots of bone resorption marker (serum CTX) levels (A) and of bone formation marker (serum PINP) levels (B) at baseline and at day 5 according to serum sodium at day 5. *Mann-Whitney test* was implemented to detect differences between the groups.

The bone formation index PINP/CTX remained stable in patients with correction of hyponatremia (p= 0.69), whereas it significantly decreased in patients with persistent hyponatremia (p= 0.01), with a difference between the two subgroups (p= 0.028), as displayed in *Figure 2.*

Figure 2 – Bone Formation Index



Boxplots of bone formation index (PINP/CTX) levels at baseline and at day 5 according to serum sodium at day 5. *Mann-Whitney test* was implemented to detect differences between the groups.

The multivariable linear regression model confirmed that the difference in the dynamic of bone formation index between patients with correction versus patients with persistency of baseline hyponatremia was independent of age, sex, BMI, smoking status, baseline cortisol levels, and intervention arm. In detail, in patients with correction of baseline hyponatremia the difference between bone formation index at discharge and baseline was on average 47.9 points higher than in patients with persistency of hyponatremia (95% CI 17.0 to 78.7, p= 0.003) (*Table 2*).

Table 2 – Results of Linear Regression Model

Independent Variables	Estimate Regression Beta Coefficient	95% CI 2.5% to 97.5%	p
Normonatremia at day 5	47.9	17.0 to 78.7	0.003
Age	-0.4	-1.6 to 0.8	0.516
Sex Female	-12.9	-43.8 to 18.0	0.404
BMI	2.9	-0.6 to 6.4	0.098
Smoking Status yes	1.6	-37.4 to 40.6	0.933
Intervention with SGLT2-Inhibition	-24.44	-55.1 to 6.2	0.115
Baseline Serum Cortisol	-0.009	-0.08 to 0.06	0.799

Linear regression model with bone formation ratio difference between baseline and day 5 as dependent variable (PINP/CTX at day 5 - PINP/CTX at baseline). CI= confidence interval; BMI= body mass index; SGLT2 = sodium-glucose co-transporter 2; PINP= N-terminal propeptide of type I collagene; CTX= C-terminal telopeptide.

This effect remained stable when adjusted additionally for use of thiazide or loop diuretics (estimate beta regression coefficient 48.2, 95% CI 14.7 to 81.7, p=0.006).

Copeptin remained stable in the two subgroups of patients with (p= 0.9) and without correction of baseline hyponatremia (p= 0.8), with no difference between the two subgroups (p= 0.9).

Discussion

In this pre-planned secondary analysis of our randomized controlled study using SGLT-2 inhibitors for treatment of hyponatremia in hospitalized patients with SIAD[136], we showed that serum bone markers and their dynamic differ significantly in patients with persistency versus patients with correction of baseline hyponatremia. In detail, patients with correction of baseline hyponatremia have an increase in osteoblast activation marker PINP measured at day 5, whereas PINP does not increase in patients with persistency of baseline hyponatremia. This produces a different dynamic in bone formation index, which is at day 5 on average 47.9 points

higher in patients with correction as compared to patients with persistency of baseline hyponatremia, independent form age, sex, BMI, smoking status, baseline cortisol levels, and intervention arm.

So far, the effect of hyponatremia on bone has been widely described as promoting osteoclast activation, both in animal models and cell cultures[40,133–135]. One would therefore expect that correction of hyponatremia would primarily reduce bone resorption. Our data however show that correction of hyponatremia in hospitalized patients mainly increase osteoblast function with no strong effect on osteoclast activation. In detail, PINP was increasing in patients with correction of baseline hyponatremia, and it was stable in patients with persistency of baseline hyponatremia, whereas CTX was increasing in both groups. As a consequence, bone formation index (PINP/CTX ratio) was decreasing from baseline to day 5 of hospitalization in patients with persistent hyponatremia, whereas it was not decreasing in patients with correction of baseline hyponatremia. Of note, this occurred despite the fact that patients with persistent hyponatremia showed a slightly higher bone formation index at baseline than patients with later normalization of hyponatremia.

So far, data about effect of correction of hyponatremia on bone markers in humans are very limited. As many studies have shown an osteoclast activation in relation to hyponatremia, one would expect that correction of hyponatremia might reverse this effect, but this was not clearly shown in humans so far. In 2018 Verbalis et al. investigated the bone markers osteocalcin and urine N-telopeptide (NTX)-creatinine ratio after 21 days of treatment with tolvaptan or placebo[54]. Although no direct effect could be shown on the osteoblast marker osteocalcin nor on the osteoclast marker NTX-creatinine-ratio, most likely due to the limited number of patients studied, they found a significant decrease in bone resorption index in tolvaptan-treated patients as compared to placebo. Their results therefore suggest that an intervention to correct hyponatremia positively influence bone metabolism, but could not clarify whether correction of hyponatremia influences osteoblast or osteoclast activation, or both. To the best of our knowledge, our study is therefore the first one showing a positive effect of hyponatremia

correction on osteoblast activation. One possible explanation for our results showing primarily an increase in PINP as a marker for osteoblast activation could be that correction of hyponatremia reduces reduces the promotion of osteoclastogenesis at expense of osteoblastogenesis, as described in cell cultures[40]. Cells growing in hyponatremic medium had shown a differentiation towards osteoclasts instead of osteoblasts, whereas this was not seen in cells cultured in normonatremic medium.

Another possible explanation for the increase in PINP could be linked to the previously described effect exerted by arginine vasopressin (AVP) on bone cells. Tamma et al. showed in a mice model that AVP exerts an inhibitory effect on osteoblasts[41]. Consequently, correction of hyponatremia might decrease AVP and therefore mitigate this inhibitory effect, allowing osteoblast activation. However, arguing against this hypothesis, in our study, we did not find a difference in copeptin levels, mirroring AVP concentration. Data from out-patients would be helpful to clarify whether correction of hyponatremia and therefore of AVP could have a positive impact on bone metabolism.

The fact that we did not see an effect on bone resorption marker CTX could be due to our population of hospitalized patients. It is well known that hospitalized patients experience immobility, which is strongly associated with bone catabolism[141,142]. In our cohort, this was mirrored by an increase of CTX in both patients with correction of baseline hyponatremia and patients with persistent hyponatremia. We therefore postulate that the strong effect of immobilization might have overruled the possible effect of hyponatremia correction on osteoclasts. The effect on bone metabolism might therefore be even stronger in patients without immobility. Analysis of data from outpatients will be helpful to better understand the influence of hyponatremia correction on bone resorption. Moreover, it would be interesting to perform a subgroup analysis in patients with correction of hyponatremia in order to understand whether the timing of sodium normalization influences bone parameters. Unfortunately, due to the limited number of patients, the present analysis had not enough power to clarify this point.

Importantly, the linear regression model showed that our results were not affected by well-known factors influencing bone metabolism, such as age, sex, BMI, and smoking status[143–146], as these factors were included as confounders. Recently, it was shown that acute hyponatremia increases bone catabolism and negatively influences bone formation index in patients with subarachnoid hemorrhage[53]. However, baseline cortisol levels were identified as a strong confounder. It is therefore important to mention that the positive effect of correction of hyponatremia on bone turnover in our study was independent from baseline cortisol levels.

A further important point is the role of SGLT2-inhibitors in correction of hyponatremia and their effect on bone metabolism. In the past years, an association between SGLT-2 inhibitors and a decrease in bone mass with increase in fracture rate[147,148] was described. The presence of a reactive hyperaldosteronism with a consequent hyperparathyroidism was suggested as a possible mechanism for the association between use of SGLT2-inhibitors and a decrease in bone mass[149]. Although we did not measure aldosterone directly, we included the use of SGLT2-inhibitors as confounder in our analysis, and could not find a negative effect of SGLT2-inhibitors on bone metabolism. A reason for that might be the exposition of merely 4 days to this drug, which could be a too short period to produce a relevant hyperaldosteronism with consequent hyperparathyroidism. Our results are in line with two meta-analyses from 2016 and 2019 that could not confirm the association between use of SGLT2-inhibitors and a decrease in bone mass[150,151]. Moreover, the association between use of SGLT2-inhibitors and hyperaldosteronisms have been described after several weeks of exposition to this drug, whereas it has not been seen after 4 day treatment[152].

Our original study had shown that SGLT2-inhibitors increased sodium levels to a higher extent compared to placebo in hospitalized patients with SIAD[136]. Therefore, one could argue that the use of SGLT2-inhibitors and the normonatremia at day 5 could be two interrelated variables, as the administration of a SGLT2-inhibitor helped in achieving normonatremia. However, this multicollinearity was ruled out by our VIF analysis.

Another important point is the use of diuretics. Many studies, mostly retrospective, have described an association between use of thiazide diuretics and improvement of bone mass index, mainly due to a reduction of urinary calcium excretion caused by thiazide diuretics[153,154]. Other studies have described an association between loop diuretics and decrease in bone mass index, attributing it to an increase in urinary calcium excretion[155,156]. We therefore included the use of thiazide and loop diuretics as confounders in our linear regression model, and both variables showed no influence on the positive effect of correction of hyponatremia.

Our study has several limitations. First, as it was a pre-planned secondary analysis of a previous randomized controlled study[136], we only included an observation period of 5 days, so we cannot draw conclusions over the long term effects of correction of hyponatremia on bone metabolism, or about medium or long term effects such as osteodensitometric changes or one-year fracture rate. However, it is very interesting to notice that even in such a short period and in hospitalized patients, correction of hyponatremia exerts a positive effect on osteoblast activation. Another limitation is that we did not measure vitamin D levels of patients. However, patients were very homogeneous for baseline characteristics and calcium levels, and had no renal or hepatic insufficiency, therefore making a significant difference in vitamin D levels unlikely. In addition, we did not investigate other bone markers such as parathyroid hormone, osteocalcin, and sclerostin. However, we chose PINP and CTX as they are considered the main markers for bone formation and bone resorption[43] . Third, our cohort is of limited size and the study was monocentric. In addition, we did not have a control group of hospitalized normonatremic patients. Nevertheless, to the best of our knowledge this is the largest study investigating the possible impact of correction of hyponatremia on bone metabolism. A strength of our study is that we had a homogeneous group of patients with SIAD induced hyponatremia undergoing a treatment whose goal was to achieve normonatremia. Moreover, to minimize possible bias we excluded patients with fractures, bone metastasis or steroid therapy, factors well-known for modifying bone metabolism markers[157–159].

In conclusion, our predefined post-hoc analysis shows that correction of hyponatremia in hospitalized patients with SIAD might have a positive impact on bone turnover, and that this correction seems to affect osteoblast function. Interventional studies with a longer follow-up, preferably on out-patients, are needed to clarify the long-term effect of hyponatremia correction on bone.

Declaration of interest: The authors disclose no conflict of interest

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Clinical Trial Information: ClinicalTrials.gov no (Number: NCT02874807)

Discussion of the PhD-Project

This PhD-project has two main results: first, persistency of admission hyponatremia and new-onset hyponatremia during hospitalization are associated with a worse clinical outcome in hospitalized patients with pneumonia and stroke, as compared to patients with normonatremia at discharge; second, active correction of hyponatremia in hospitalized patients with SIAD is associated with an increase in osteoblast activation.

In the past decades, many studies have documented an association between admission hyponatremia in hospitalized patients and worse clinical outcomes, independent from underlying disease[30,55,77]. The first and second study of this project provide evidence that not only admission hyponatremia, but also persistency of hyponatremia or presence of hyponatremia at discharge are associated with a worse outcome measured at medium range (6 months for pneumonia, 3 months for stroke). This association is not present in patients with normalization of initial hyponatremia, suggesting a possible positive impact of correction of hyponatremia on patient outcomes.

Normalization does not mean correction, and one could argue that normalization of hyponatremia occurred only in patients who had completely recovered from the disease causing hyponatremia and hospitalization, whereas patients with persistency of hyponatremia had not, and therefore patients with persistency of hyponatremia experienced a worse outcome in the short and medium time follow-up. This approach suggests that hyponatremia might be a valuable marker for illness and for severity of underlying disease(s), but not a factor direct influencing patients' welfare. According to it, a goaled correction of hyponatremia would not play an active role in improving patients' clinical outcome.

However, in our analysis hyponatremia at discharge predicted a worse outcome independent from pneumonia-severity index and comorbidities in patients with pneumonia, and remained stable after adjusting for NIHSS- and mRS score on admission as well as comorbidities in

patients with stroke, strongly suggesting an independent role of hyponatremia in patient outcomes.

This independent role of hyponatremia on patient outcomes is indirectly suggested by different studies showing that hyponatremia causes symptoms not related to underlying disease, not only in acute setting, when the fall of serum sodium level comes suddenly, but also in patients experiencing hyponatremia over several months[126,160,161]. Presence of hyponatremia has been widely associated with a cognitive impairment, attention deficit, gait disturbance, and increased risk of death, rehospitalization, falls, and fractures[131,162].

Pathophysiologically, as hyponatremia means a change in ECF composition, with an alteration of sodium and water homeostasis, it also causes an alteration of the homeostasis between cells and ECF. In order to restore homeostasis, cells react by altering first their volume and then their osmolytes (e.g., myoinositol, betaine, glutamine, taurine, and g-aminobutyric acid etc.) composition. A recent analysis demonstrated that in chronic hyponatremia (>48 h), loss of both electrolytes and organic osmolytes from brain cells represents a very important mechanism to counterbalance the potential brain swelling caused by hypotonicity and hyponatremia[163].

Moreover, a review from Portales-Castillo and Sterns in 2019 went through adaptive responses of different human cells during hyponatremia and gave an insight of what such an adaptation could mean in terms of dysfunction[82]. The authors pointed out at the loss of taurine from leucocytes, other blood cells and cardiomyocytes, as well as at the loss of glutamate from brain cells, in order to keep cell volumes. In the first two presented studies, a clear association between persistency of hyponatremia and worse patients' outcome as compared to normonatremia could be shown, suggesting an active role of hyponatremia on patients' health. Patients with persistency of hyponatremia and pneumonia showed an increased risk of recurrence up to 6 months after index hospitalization, and patients with persistency of hyponatremia and stroke showed a worse functional outcome measured by the modified Rankin Scale at 3 months. Both of these studies suggest that the adaptation to hyponatremia

undergone by the immune cells respective the cerebral cells might have negatively influenced cell function and performances. Moreover, they emphasize the hypothesis that hyponatremia plays an active role in causing pathological alterations, independent from the underlying disease, by directly worsening cell functions as a result of the needed adaptation to maintain osmotic and volume homeostasis. Based on this knowledge, it can be postulated that correction of hyponatremia might reverse these changes.

A direct effect of hyponatremia on bone cells homeostasis and a reversibility of this effect was strongly suggested by a case report from 2014[32]. The authors described how a young man recovered from osteoporosis after excision of a SIAD producing tumor causing hyponatremia, whereas antiresorptive therapy was failing for several months in treating osteoporosis. The effect of hyponatremia on bone metabolism seems to be primarily mediated by promotion of osteoclast activation[39,40,135]. As pointed out by Portales-Castillo, osteoclast activation with consequent increase of bone resorption and decrease in bone formation represents an important adaptation to hyponatremia, as it allows mobilization of bone sodium to preserve ECF volume[82]. The third and main study of this PhD-project is the first prospective clinical trial providing evidence that a goaled and effective correction of hyponatremia might reverse the hyponatremia induced mismatched between bone resorption and bone formation. Our analysis demonstrated that correction versus persistency of SIAD induced hyponatremia in hospitalized patients results in an increase in osteoblast activation, independent from underlying disease causing hospitalization and other well-known factors influencing bone metabolism such as age, sex, BMI, smoking status, and baseline cortisol levels. Specifically, osteoblast activation marker PINP increased in patients whose hyponatremia was corrected, whereas it remained stable in patients with persistency of hyponatremia, showing that osteoblast function was improving in patients with correction of hyponatremia as compared to patients with persistency of hyponatremia.

These results are only apparently in contrast with those from a trial published in 2020 investigating the effect of an intervention to treat hyponatremia on serum BTM levels[164]. In their

study, Diemar et al. performed a three-month intervention with salt supplementation +/- furosemide versus placebo in patients with hyponatremia and epilepsy and found no difference in serum PINP and CTX levels between the 2 groups. However, this study was performed in a limited number of patients (14 interventions, 7 controls), and no difference could be found in the rate of correction of hyponatremia between intervention and control group (p= 0.706), with almost 100% of participants reaching normonatremia at primary endpoint, so no assessment could be made about the effect of correction versus persistency of hyponatremia on BTM levels. On the contrary, in our study we could compare 41 patients with persistency of initial hyponatremia vs 27 patients with correction of initial hyponatremia at day 5 and we found a difference in PINP levels after adjusting for age, sex, BMI, smoking status, and baseline cortisol levels, as described above. In addition, in the study from Dieter et al. a difference in serum sodium levels between intervention and control group could be found at visit 3 (two weeks after study inclusion), and a difference in PINP level between intervention and control could actually be detected at that time-point (median (IQR) intervention vs control 34.3 (29.5-40.1) vs 50.0 (38.8-64.9) mcg/l, p= 0.056).

Of note, in our study osteoclast activation marker CTX was increasing both in patients with correction and persistency of hyponatremia. As a result, PINP/CTX ratio as a marker for bone formation decreased in patients with persistency of hyponatremia, whereas it slightly increased in patients with correction of hyponatremia. In 2020, Garrhay et al.[53] documented a decrease in bone formation defined as PINP/CTX ratio in subjects developing acute hyponatremia. One can therefore conclude that our study indirectly showed a reversibility of this effect after correction of hyponatremia.

The fact that we did not see an effect on CTX and therefore on osteoclast function might be due to our population of hospitalized patients. As a matter of fact, hospitalized patients are often immobilized. As immobilization is a well-known strong stimulus for osteoclast activation[165,166], this strong stimulus might have overridden the beneficial effect of correction of hyponatremia.

Taken together, this PhD-project strongly suggests an independent, causal role of hyponatremia in negatively influencing patients' clinical outcome and bone metabolism, and therefore that a targeted correction of hyponatremia might represent an important additional therapeutic approach in hospitalized patients with pneumonia and strokes, as well as an additional measure in preventing osteoporosis.

Directions for future research

Hyponatremia is a high prevalent finding in hospitalized patients. On one hand, it leads to symptoms both in acute and in chronic setting, on the other hand *in vitro* and *in vivo* studies have shown an altered cell homeostasis in response to hyponatremia, resulting in functional impairment.

In the past years, many studies have described an association between admission hyponatremia and poor clinical outcome in hospitalized patients[77,80,131], and some studies have shown this same association in ambulatory patients[2]. This PhD-project shows that persistency and new-onset hyponatremia in hospitalized patients are associated with a poorer clinical outcome as compared to normonatremia. Interventional studies with an active correction of hyponatremia versus no correction and important clinical outcomes as endpoints are needed to verify whether association means causality. So far, there is no completed trial in humans addressing this issue, but an ongoing, investigator initiated, international trial, which I have the pleasure to participate as a study physician to (EKNZ 2018-00971, NCT 03557957)[56]. Evidence of a causal connection between hyponatremia and poor patients' outcomes and of the possible clinical improvement after correction of hyponatremia would have a strong clinical impact. It would change the approach to hyponatremia and potentially establish hyponatremia correction as an additional treatment for improving the prognosis of hospitalized patients, and eventually also of ambulatory ones.

As regards bone metabolism, this PhD-project showed a direct short-term impact of correction of hyponatremia on bone metabolism. Interventional clinical trials with a longer follow-up could clarify on one hand the changes in fracture incidence according to correction versus persistency of hyponatremia, and on the other hand the effect of hyponatremia correction on bone architecture and structure. In addition, the use of serum bone markers can help understanding changes of bone metabolism in relation to hyponatremia and its correction without using invasive methods such as biopsies, or serial bone density measurements with consequent radiation exposure.

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Curriculum Vitae

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WORK EXPERIENCE

09/2021 ongoing	Senior Research Physician University Hospital of Basel, Division of Endocrinology, Diabetology and Metabolism, Basel, Switzerland (50% position)
08/2021 ongoing	Senior Physician cantonal hospital of Baselland, Division of Endocrinology and Diabetology, Liestal, Switzerland (50% position)
09/2018 – 11/2021	MD-PhD Clinical Research and resident physician University Hospital of Basel, Division of Endocrinology, Diabetology and Metabolism, Basel, Switzerland

06/2012 – 05/2018	Resident physician, Division of Internal Medicine, Endocrinology, Gastroenterology and Infectious Medicine, University Hospital of Freiburg, Germany
08/2011 – 12/2012	Medical Research physician, Division of medical physiopathology and general surgery, University Hospital of Rome, Italy

RESEARCH EXPERIENCE

09/2018 ongoing	PhD, clinical research, University Hospital of Basel, Switzerland *Topic:* Influence of Hyponatremia on inward patients' clinical outcome with focus on bone
06/2012 – 05/2018	Research assistant in the working group of Professor J. Seufert, University Hospital of Freiburg, Germany *Topics*: gestational diabetes mellitus; obesity
10/2010 – 01/2011	MD thesis with Professor A. Fantoni, University Sapienza of Rome, Italy *Topic:* SNP of TCF7L2 and gestational diabetes mellitus
05/2010 – 07/2012	Student research assistant in the working group of Professor J. Seufert, University Hospital of Freiburg, Germany. Supervision for MD Thesis by Professor Fantoni from Sapienza University of Rome, Italy
05/2008 – 05/2010	Student research assistant in the working group of Professor F. Consorti, University Sapienza of Rome, Italy

CERTIFICATION

Since 2015	GCP refresh course, last in 01/2021
01/2021	Course Safety Management in Clinical Research, University of Basel, Switzerland

09/2019	Good Clinical Practice (GCP) Advanced course Sponsor-Investigator level, University of Basel, Switzerland
06/2019	Approval as Specialist for Endocrinology and Diabetology in Switzerland
12/2018	Approval as Physician in Switzerland
11/2018	Approval as Specialist for internal medicine and Endocrinology in Baden Württemberg, Germany
11/2013	Good Clinical Practice (GCP) Basic course Investigator level, University of Freiburg, Germany
05/2012	Approval as Physician in Germany – Baden Württemberg
07/2011	Approval as Physician by the Italian government

EDUCATION

11/2004 – 01/2011	Medical School, University Sapienza of Rome
2009	Exchange Student (Erasmus), University of Würzburg, Germany
10/2001 – 11/2004	Bachelor degree in Philosophy at University LaTerza of Rome, Italy
09/1996 – 07/2001	High School, Rome, Italy Final Exam: Abitur

MEMBERSHIP IN BOARDS AND SCIENTIFIC SOCIETIES, SCIENTIFIC REVIEWING ACTIVITIES

2020- ongoing	Editorial Board of European Journal of Internal Medicine
2019- ongoing	European Endocrine Society (ESE)
2019- ongoing	Swiss Society of Endocrinology & Diabetology (SGED)

MEMBERSHIP IN PANELS

2018- ongoing	Present Swiss Association of Residence and Consultants (VSAO/ASMAC), Switzerland. www.vsao.ch
2012-2018	Membership Medical Chamber Baden-Württemberg, Germany
2011-2012	Membership Medical Chamber Rome, Italy

PRIZES, AWARDS AND FELLOWSHIP

2021	Meeting Grant to attend the annual European Congress of Endocrinology (e-ECE).
2018-2021	MD-PhD-project support – Alumni Medizin Basel, Switzerland
2018	University Pool for Research, University of Basel, Switzerland
2010	Scholarship for Research for MD Thesis at University of Freiburg, Germany
2009	Scholarship for Erasmus Program, University Sapienza of Rome, Italy

Publications

Potasso L, Refardt J, Meier C, Christ-Crain M. Effect of Hyponatremia Normalization on Osteoblast Function in Patients with SIAD, European Journal of Endocrinology 2021 (published online ahead of print 2021), EJE-21-0604.

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