Apelin, copeptin, and salt-water balance-related laboratory and body composition observations in humoral regulation disorders

Doctoral (Ph.D.) thesis

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ABBREVIATIONS

ACE: angiotensin-converting enzyme

ACTH: adrenocorticotropic hormone

ADD: antidepressant drug

ADH: antidiuretic hormone

AEP: antiepileptic drug

ANH: atrial natriuretic hormone

APACHE II: Acute Physiology and Chronic Health Evaluation II

APD: antipsychotic drug

AQP2: aquaporin-2

ARB: angiotensin receptor blocker

AVP: arginine vasopressin

BIA: bioelectrical impedance analysis

BMI: body mass index

CAD: coronary artery disease

cAMP: cyclic adenosine monophosphate

COVID-19: coronavirus disease 2019

CRH: corticotropin-releasing hormone

CT-proAVP: C-terminal pro-arginine vasopressin

DTC: differentiated thyroid cancer

ECOG: Eastern Cooperative Oncology Group

ECW: extracellular water

EDTA: ethylenediaminetetraacetic acid

eGFR: estimated glomerular filtration rate

ELISA: enzyme-linked immunosorbent assay

fT3: free triiodothyronine

fT4: free thyroxin

GFR: glomerular filtration rate

HIF-1: hypoxia-inducible factor 1

HPA: hypothalamic-pituitary-adrenal

ICU: intensive care unit

ICW: intracellular water

LPS: lipopolysaccharide

MAOI: monoamine oxidase inhibitor

MRI: magnetic resonance imaging

NSAID: nonsteroidal anti-inflammatory drug

NT-proBNP: N-terminal prohormone of brain natriuretic peptide

PEG: percutaneous endoscopic gastrostomy

RAI: radioiodine

RAS: renin-angiotensin system

SAPS II: Simplified Acute Physiology Score II

SD: standard deviation

SIAD: syndrome of inappropriate antidiuresis

SIADH: syndrome of inappropriate antidiuretic hormone secretion

SSRI: selective serotonin reuptake inhibitor

TBW: total body water

TCA: tricyclic antidepressant

TRH: thyrotropin-releasing hormone

TSH: thyroid-stimulating hormone

V2-R: vasopressin-2 receptor

PROLOGUE

In the complex network of human physiology, the conscious influence of the delicate balance between health and disease depends on a deep knowledge of the complex interaction between many biological systems, thus humoral homeostasis. The mainstream of medical research seeks to unravel the mysteries behind diseases in order to develop future diagnostic and therapeutic approaches by understanding the underlying mechanisms. The three topics discussed in the thesis - short-term severe hypothyroidism, humoral changes in critical illness, and drug-induced hyponatremia - are instructive examples of the complexity of human health and disease.

SECONDARY HORMONAL ALTERATIONS IN SHORT-TERM SEVERE HYPOTHYROIDISM; IN THE FOCUS: APELIN AND COPEPTIN

The effects of short-term severe hypothyroidism, a condition that impacts the endocrine system, are explored in this chapter. The synchronized dance of physiological processes, which is coordinated by hormones, is disturbed in the absence of thyroid hormones, which has a variety of negative effects. Our study aimed to investigate the very intricate interplay between humoral variables in fluid-ion homeostasis in patients with short-term severe hypothyroidism.

APELIN-13 AS A POTENTIAL BIOMARKER IN CRITICAL ILLNESS

The body's homeostasis is upended during critical illness, marking a crucial turning point that calls for a greater comprehension of the humoral alterations that result. This chapter examines the complex communication channels that influence diagnosis and prognosis, focusing on the coordinated reactions of hormones and biochemical parameters during times of crisis. Our present study aimed to investigate a more complex interplay of the hypothalamic and adrenocortical systems in a mixed population of patients with critical illness and to analyze their potential prognostic values.

HUMORAL AND BODY COMPOSITION INVESTIGATIONS IN HYPONATREMIA-RELATED DISORDERS

Therapeutic interventions often have unforeseen repercussions, which makes modern medicine a double-edged sword. Laboratory tests and body composition assessments clarifies the interaction between drugs, electrolytes, and physiological processes.

This chapter explores the serious consequences of drug-induced hyponatremia, a possible side effect of pharmacological therapy, and emphasizes the need for prudent pharmacological management.

This thesis examines where biochemical parameters and hormonal cascades interact, serious illness pushes the edge of human adaptability, and therapeutic medications may compromise the delicate electrolyte balance. By shedding light on the disease's underlying mechanisms, this effort attempts to demonstrate how to improve diagnostic and therapeutic strategies.

SECONDARY HORMONAL ALTERATIONS IN SHORT-TERM SEVERE HYPOTHYROIDISM; IN THE FOCUS: APELIN AND COPEPTIN

INTRODUCTION

Hyponatremia is the most common electrolyte disorder in hospitalized patients, which occurs when the plasma sodium concentration falls below 135 mmol/L (1). Various underlying conditions have been associated with hyponatremia, including chronic heart failure, chronic kidney disease, liver cirrhosis, hypovolemia, and the Syndrome of Inappropriate Antidiuresis (SIAD) (1, 2). Hypothyroidism can cause fluid-electrolyte imbalances that may result in hyponatremia, but the exact mechanism and prevalence are not fully understood. Antidiuretic hormone (ADH)/arginine vasopressin (AVP) is released in response to increasing plasma osmolality or various stressors, such as hypovolemia, hypoxia, acidosis, or severe infections (3). Even in reduced serum osmolarity, hypovolemia remains the primary stimulus for ADH/AVP secretion, which acts on vasopressin-2 receptor (V2-R) to increase cAMP production and aquaporin-2 (AQP2) insertion into the apical membrane of the collecting duct in the kidney, leading to water reabsorption. Non-osmotic elevation of ADH/AVP has been associated with cardiac fibrosis and dysfunction (4, 5).

Some researchers found that thyroid hormone supplementation helped restore hypothyroid patients' high levels of ADH/AVP (4, 6). Others, however, discovered decreased ADH/AVP in patients with myxedema (7), explaining hyponatremia with an ADH-independent mechanism of reduced water excretion. This may be the outcome of renal impairment brought on by hypothyroidism, with a reduced GFR and altered tubular activity (4, 8, 9). Methodological issues may contribute to these contradictory findings because measuring ADH/AVP requires competitive assays due to its small size and is less accurate than measuring copeptin (10). ADH/AVP is primarily linked to platelets in the blood (11), and the long-term storage of unprocessed blood samples could inadvertently increase the levels of ADH/AVP (10, 11). Additionally, aliquots of ADH/AVP kept at -20°C or -80°C become unstable (12). As a result, routine monitoring of circulating ADH/AVP levels in patient treatment was never possible (10). While copeptin is a humoral marker of ADH/AVP production that is more accurate (10), its changes in hypothyroidism have not been studied.

The altered production of additional humoral components may also contribute to the development of hyponatremia in SIAD and the elevation of copeptin levels.

For instance, compared to healthy persons, the sex- and age-adjusted plasma levels for apelin and copeptin in SIAD patients are 26% and 75% higher, respectively (1, 13). In 86% of patients with SIAD, the plasma apelin/copeptin ratio is outside the expected range. This finding highlights the primary osmoregulatory defect in these patients. Hyponatremia is worsened by an inappropriately low plasma apelin concentration that cannot offset the elevated ADH/AVP release (13).

In both animal and human models, the plasma osmolality controls apelin release from the hypothalamus in the opposite way to how it controls ADH/AVP (14). Particularly abundant apelin colocalizes with ADH/AVP in magnocellular neurons in the hypothalamic supraoptic and paraventricular nuclei (15, 16, 17, 18), preventing the secretion of ADH/AVP. By boosting renal blood flow and reversing the antidiuretic effects of ADH/AVP on the distal convoluted and collecting tubules of the kidney, apelin increases aqueous diuresis (1, 19). As a result, apelin appears to be essential for preserving fluid balance. Lower apelin levels may block ADH/AVP secretion and activity.

Under physiological circumstances, the magnocellular ADH/AVP neurons release ADH/AVP and apelin in an appropriate ratio to the current plasma osmolality (1). After water deprivation, ADH/AVP is depleted in magnocellular neurons because it is released from magnocellular vasopressinergic neurons into the bloodstream more quickly than synthesized. In the interim, apelin production declines and builds up in magnocellular neurons. Consequently, following dehydration, apelin and ADH/AVP are controlled in opposing ways to promote systemic ADH/AVP release and prevent diuresis.

During water loading, apelin release increases, depleting apelin in magnocellular neurons, while ADH/AVP release decreases from magnocellular vasopressinergic neurons, resulting in a buildup of ADH/AVP. ADH/AVP and apelin are controlled in different ways following water loading to allow systemic apelin release and boost aqueous diuresis.

Preproapelin, the precursor to the peptide hormone apelin, is transcribed at different sites throughout the central nervous system, with the thalamus and frontal cortex exhibiting the highest quantities (16, 20, 21, 22). Moreover, apelin and/or its receptor are present in the placenta, heart, lung, kidney, liver, gastrointestinal system, adrenals, uterus, and ovaries, among other tissues (23, 24, 25, 26), endothelial cells of various vessels, and plasma cells (27, 28, 29). In addition, apelin expression rises during adipocyte differentiation (30).

Apelin has a positive inotropic effect by increasing cardiac output and is an essential regulator of blood pressure dependent on the endothelium (19, 31). Hypoxia induces the release of HIF-1, which increases the signaling of the apelinergic system. However, it is unknown how hypovolemia affects the apelinergic system on its own (1, 31).

The decreased level of atrial natriuretic hormone (ANH), paradoxically opposite to the extracellular volume expansion, is another humoral change in hypothyroidism (4, 32). Additionally, hypothyroid patients frequently have lower plasma renin activity and plasma aldosterone concentrations (4), which can be explained as a humoral response to extracellular fluid retention.

OBJECTIVES

Our research sought to learn more about the complex interactions between these humoral factors that affect fluid-ion balance in people with severe transitory hypothyroidism.

MATERIALS AND METHODS

STUDY DESIGN

After informed consent was gained, venous blood samples for biochemical investigation were taken between 8 and 10 in the morning. After that, body weight was measured to the nearest kilogram and height to the nearest centimeter. The participant was asked to stand up straight while the tape measure measured the waist circumference in the horizontal plane halfway between the lowest rib and the iliac crest and the hip circumference over the most prominent area of the buttocks. After entering collected data in the BIA device, body composition was measured. To eliminate inter-individual variations, the same researcher took all the anthropometric measurements.

We received approval from the University of Pécs Regional Research Ethics Council (6961/2017) to conduct our study in accordance with the Declaration of Helsinki ethical principles from 2003. Participants or the participants' parents, legal guardians, or next of kin provided written informed consent before participating in the study.

PATIENTS

Patients with differentiated thyroid cancer (DTC) were included between the ages of 18 and 75. In this prospective, observational study, patients who underwent total or nearly total thyroidectomy and had no metastases were scheduled for radioiodine (RAI) therapy. From 12 January 2018 to 21 February 2020, the patients were enrolled.

Before being sampled, they had endured a complete endogenous or exogenous levothyroxine deficiency for at least four weeks. Low serum free thyroxine (fT4), low serum free triiodothyronine (fT3), and noticeably high thyroid-stimulating hormone were used to confirm hypothyroidism in all individuals (TSH). Patients were prospectively assessed before starting RAI therapy and again 10–12 weeks after starting thyroxine. DTC wasn't developed (ECOG: 0). Any known chronic conditions or medications, including abnormal fluid retention brought on by heart, liver, or kidney conditions or treatment with diuretics, were prohibited. There were no notable intercurrent diseases between the hypothyroid and control studies, nor were there any modifications in the drugs other than the addition of thyroxine. Patients took regular medications, including levothyroxine supplementation, before control laboratory tests in the morning during the 10–12 weeks of follow-up.

Determination of body composition, routine laboratory tests, and neurohormonal mediators

Body composition was evaluated using a bioelectrical impedance analysis (BIA) device (Bodystat Quadscan 4000, Bodystat Ltd., P.O. Box 50, IM99 1DQ Douglas, Isle of Man, United Kingdom). Before the measurement, patients were laid out in the supine position for at least five minutes. All participants wore light clothing and removed earrings, rings, bracelets, and any other metal which could influence the measurement results. Every measurement took each participant about 30 seconds.

Only patients who complied with the BIA protocol, which calls for abstaining from alcohol for 48 hours, refraining from strenuous activity for 12 hours, and fasting for at least four hours before the test, were accepted. Patients who had implanted electronic devices, such as pacemakers for the heart, were disqualified.

Venous blood samples were taken into plain tubes with accelerator gel and EDTA tubes (Vacutainer®, Becton Dickinson). After centrifuging sera and plasma samples for 20 min at 2300 rpm, the samples were aliquoted in Eppendorf tubes and kept at -80°C until analysis.

The Department of Laboratory Medicine at the University of Pécs (accreditation number: NAH-1-1553/2016) used standard laboratory diagnostic kits and automated instrumentation to determine the chemistry panel results, endocrine parameters, and fully automated blood picture tests. The reference range for TSH was 0.27-4.2 mU/L, for fT4 12.0-22.0 pmol/L. Serum apelin and copeptin levels were measured with the ELISA method using Human Apelin ELISA kit (Catalog No.: abx585113, Abbexa Ltd., UK, intra-assay CV<10%, inter-assay CV<10%), and Human Copeptin (CT-proAVP) ELISA kit (Catalog No.: abx252269, Abbexa Ltd., UK, intra-assay CV<10%, inter-assay CV<10%) according to the manufacturer’s instructions on a Biotek Synergy HT plate reader at 450 nm. The Immulite 2000 automated device was used to perform an immunoassay to measure the levels of serum NT-proBNP (Catalog No.: L2KNT2, LNTCM, Siemens Healthcare Diagnostics Inc., USA).

Statistical analysis

The Shapiro-Wilk test examined the normality of continuous variables' distributions. When median and interquartile values fall under the non-normal distribution, data are given as mean SD for parameters with a normal distribution.

The paired sample t-test and Wilcoxon signed-rank test were used to compare the two phases in normal and non-normal distributions, respectively. We used the Bonferroni correction of the p-values in the pairwise comparison to reduce the type I error. Correlation analysis with Spearman coefficient calculation was carried out to evaluate the strength of the link between the various variables. The correlations between clinical factors and the serum levels of copeptin and apelin were found using multiple regression analysis. In multiple regressions, the variables significantly related to the single result in the univariate analysis and those thought to be biologically plausible were considered. Statistical significance was assumed to be present at 0.05. SPSS (version 22.0, SPSS Inc, Chicago, IL, USA) was used for all analyses.

RESULTS

Thirty-nine patients in total (11 men, 28 women) with an average age of 50.28 ± 14.90 years were assessed. Table 1 displays anthropometric and biochemical characteristics before and after hypothyroidism correction.

Table 1. Comparisons of selected anthropometric and biochemical characteristics between the two study phases. Significant associations are labeled as bold.

Parameters	Hypothyroid phase	Control phase	p-value
Waist circumference (cm)	**94.1 ± 14.7**	**93.5 ± 14.5**	**0.039**
Body Mass Index	**29.1 (25.1 - 32.1)**	**28.2 (24.7 - 32.3)**	**<0.001**
Fat mass (kg)	**27.5 (21.4 - 32.3)**	**24.2 (18.0 - 31.9)**	**0.001**
Total body fluid (L)	38.3 (34.1 - 46.2)	37.8 (34.2 - 45.9)	0.624
Extracellular fluid (L)	16.8 (15.1 - 19.7)	17.3 (15.5 - 20.2)	0.673
Intracellular fluid (L)	22.3 (19.4 - 28.2)	22.4 (18.9 - 29.5)	0.451
TSH (mU/L)	**75.8 ± 25.3**	**2.5 ± 4.1**	**<0.001**
fT4 (pmol/L)	**2.9 ± 1.7**	**24.9 ± 5.7**	**<0.001**
Na (mmol/L)	**140.8 ± 2.6**	**142.3 ± 1.8**	**0.002**
Cl (mmol/L)	**100.8 ± 3.4**	**102.5 ± 2.6**	**0.012**
K (mmol/L)	**4.3 ± 0.4**	**4.4 ± 0.3**	**0.025**

eGFR (mL/min/1.73 m^2)	**77.0 (61.0 - 90.0)**	**90.0 (72.0 - 90.0)**	**<0.001**
Uric acid (umol/l)	295.3 ± 84.9	287.2 ± 91.8	0.363
Serum osmolality (mOsmol/kg)	287.5 ± 14.7	286.9 ± 7.1	0.817
Urine osmolality (mOsmol/kg)	530.8 ± 257.0	602.7 ± 253.9	0.136
Apelin (pg/mL)	**1901.9 ± 766.7**	**3080.7 ± 517.0**	**<0.001**
Copeptin (pg/mL)	1007.7 (626.0 - 1822.8)	933.9 (619.6 - 1437.4)	0.615
NT-proBNP (pg/mL)	**36.8 (20.6 - 73.7)**	**69.6 (37.4 - 194.9)**	**<0.001**
Renin activity (ng/mL/h)	**0.7 (0.4 - 1.6)**	**1.6 (0.9 - 2.9)**	**0.003**
Aldosterone (pg/mL)	166.5 (112.2 - 221.3)	174.2 (156.9 - 268.8)	0.180
ACTH (pg/mL)	**11.9 (10.0 - 16.0)**	**16.4 (12.2 - 22.5)**	**0.001**
Cortisol (nmol/L)	318.8 (242.3 - 385.9)	348.0 (275.6 - 440.2)	0.283

No patient had ascites, pleural effusion, or severe peripheral edema retention. A mean dose of 139.74 ± 31.27 µg. of thyroxine was administered to treat hypothyroidism. In the hypothyroid state, the apelin level was 38% lower than after therapy, but the copeptin levels remained the same. Serum levels of sodium, chloride, potassium, estimated glomerular filtration rate (eGFR), NT-proBNP, adrenocorticotropic hormone (ACTH), and renin activity were also considerably lower in the hypothyroid state.

During hypothyroidism, the BMI, waist circumference, and total body fat mass were all increased. Extracellular fluid, intracellular fluid, serum osmolality, and urine osmolality results did not differ. Only two patients (5.1%) with mild hyponatremia were found during the hypothyroid period; none of the euthyroid patients had the condition.

In Figures 1-3 and Tables 2-5, the relationships between apelin, copeptin, and a few other variables are depicted.

Table 2. Relations of apelin and copeptin to the selected anthropometric and biochemical parameters. Significant associations are labeled as bold.

Parameters	R values for apelin	p-value	R values for copeptin	p-value
Age	0.077	0.504	0.075	0.512
Waist circumference	0.004	0.969	0.035	0.761
Body Mass Index	-0.039	0.737	0.043	0.706
Fat mass	0.064	0.576	0.071	0.535
Total body fluid	-0.198	0.082	0.096	0.403
Extracellular fluid	-0.103	0.369	0.199	0.080
Intracellular fluid	-0.173	0.130	0.063	0.586
TSH	**-0.682**	**<0.001**	-0.066	0.568
fT4	**0.692**	**<0.001**	0.067	0.599
Na	**0.349**	**0.002**	**0.226**	**0.046**
Cl	**0.451**	**<0.001**	0.186	0.115

K	**0.271**	**0.017**	0.176	0.122
eGFR	0.175	0.125	-0.131	0.251
Uric acid	-0.173	0.130	0.142	0.216
Serum osmolality	-0.031	0.789	0.155	0.177
Urine osmolality	0.091	0.426	-0.008	0.945
Apelin	1	1	**0.331**	**0.003**
NT-proBNP	**0.390**	**<0.001**	0.012	0.914
Renin activity	0.088	0.446	0.006	0.956
Aldosterone	0.097	0.402	0.113	0.326
ACTH	0.177	0.120	0.112	0.328
Cortisol	0.037	0.744	0.045	0.692

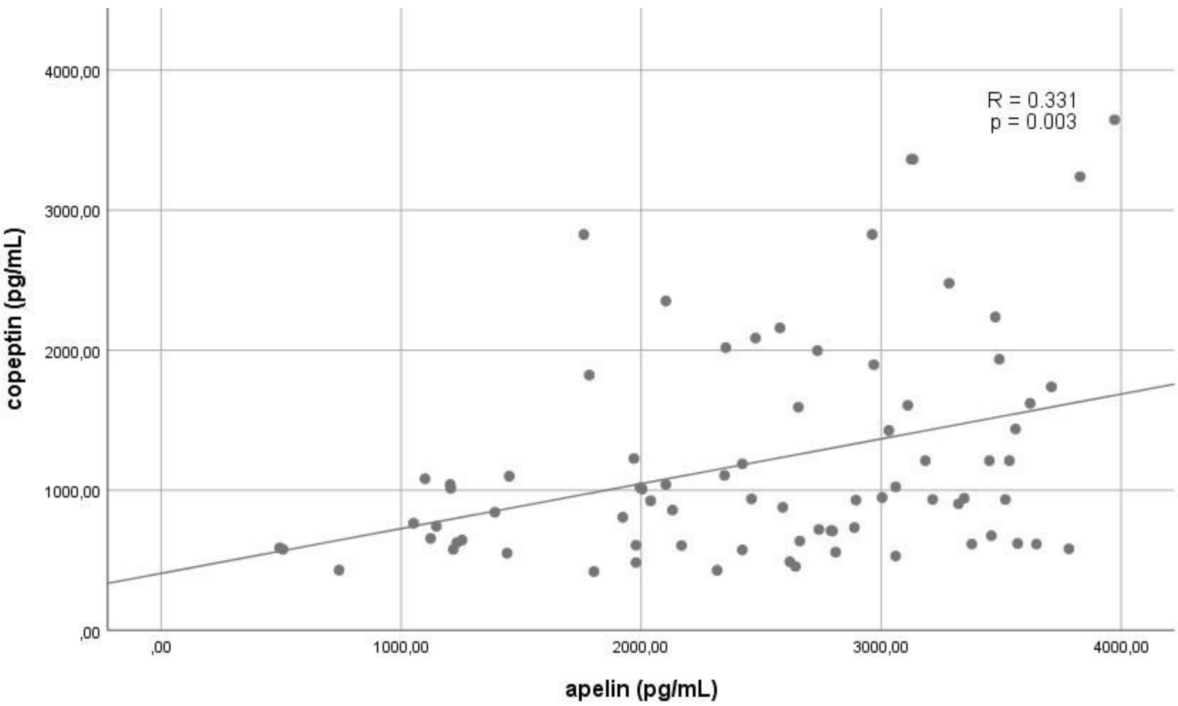

Figure 1. Correlation between copeptin and apelin.

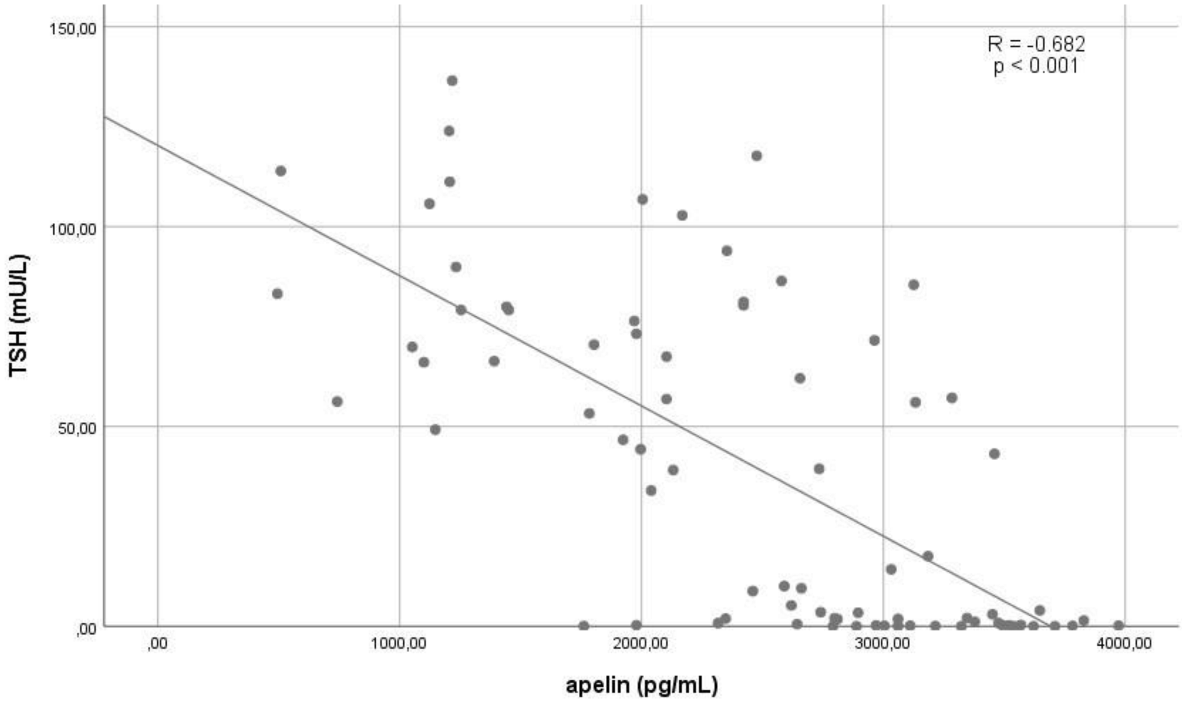

Figure 2. Correlation between TSH and apelin.

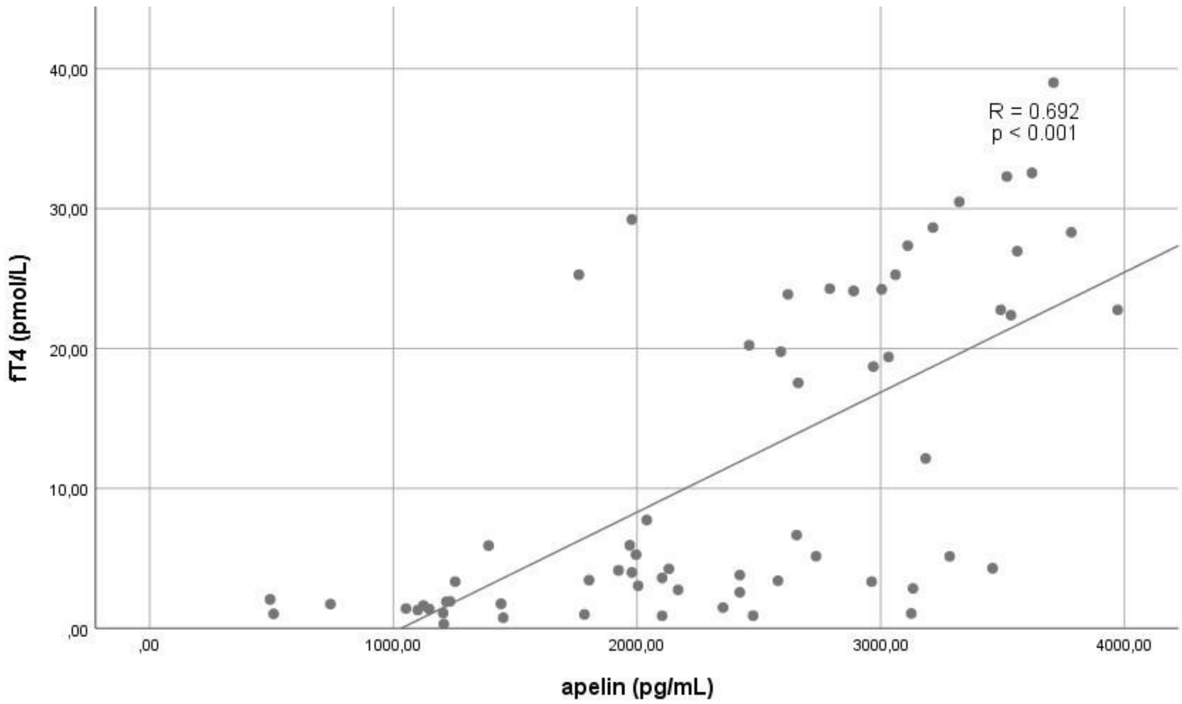

Figure 3. Correlation between fT4 and apelin.

The entire database was utilized to evaluate correlations in order to expand the scope of the researched biochemical parameters. Correlations between apelin, copeptin, and serum sodium levels were observed. While apelin had a positive correlation with sodium, chloride, potassium, NT-proBNP, and fT4 and a negative correlation with TSH, copeptin was not correlated to any other examined parameter. Apelin and copeptin did not differ in gender and were not correlated to anthropometric measurements such as BMI, fat mass, or body fluid values. In 10 patients, the regularly administered thyroxine supplementation caused fT4 levels to rise above the reference range. Beyond TSH and fT4 levels, none of the examined parameters were different in this subset from the other patients. Changes in relevant humoral factors of fluid-ion homeostasis in hypothyroidism and their correlations are summarized in Table 3.

Table 3. Summary of relevant humoral factors for fluid-ion homeostasis; changes in hypothyroidism compared to the corresponding levels after thyroid hormone replacement and their correlations. Significant values are labeled as bold.

Humoral factor	Level in hypothyroidism	R values of correlations			
		Apelin	Copeptin	NT-proBNP	Aldosterone
Apelin	**-38%**	1	**0.331****	**0.390*****	0.097
Copeptin	+7%	**0.331****	1	0.012	0.113
NT-proBNP	**-48%**	**0.390*****	0.012	1	-0.087
Aldosterone	-5%	0.097	0.113	-0.087	1
Cortisol	-9%	0.037	0.045	-0.023	**0.331****

* p-value is between 0.05 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

The potential independent determinants of serum apelin or copeptin concentrations were examined using linear regression tests. Tables 4 and 5 display the results that are the most representative.

Table 4. A linear regression model with apelin as a dependent variable.

	Predictive power (%)	p-value
(Constant)		0.575
Age	0	0.869
Sex	1.5	0.114
BMI	1.4	0.441
Na	0.8	0.306
Copeptin	**13**	**<0.001**
TSH	**48.7**	**0.007**
fT4	1.3	0.184
NT-proBNP	0	0.890

Table 5. A linear regression model with copeptin as a dependent variable.

	Predictive power (%)	p-value
(Constant)		0.582
Age	0.5	0.530
Sex	0.1	0.835
BMI	2	0.146
Na	0	0.784
TSH	**12.3**	**0.001**
Apelin	**13.1**	**<0.001**

To increase the number of evaluated parameters during the regression analysis, they were conducted on the pooled data. Serum apelin levels were significantly influenced by copeptin and TSH levels. TSH effectively explained a significant portion of the variance in apelin concentrations.

Even though there was no link between them in a univariate test, TSH was another independent predictor of copeptin levels in addition to apelin. However, apelin was a stronger predictor of copeptin concentrations than TSH (12.3% vs. 13.1%).

Apart from apelin and copeptin, the serum sodium level varied by 5.47 mmol/L and was correlated with the levels of cortisol, ACTH, and TSH ($r = -0.321$, $p = 0.004$, $r = 0.349$, $p = 0.002$, and $r = 0.225$, $p = 0.047$).

In multiple regression analyses, however, none were independent predictors of serum sodium levels.

DISCUSSION

The literature has shown conflicting findings about apelin levels in hypothyroid patients. Even though some writers did not detect a significant difference in patients with subclinical or manifest hypothyroidism (28, 33), others found that levothyroxine treatment recovered low apelin levels in subclinical hypothyroidism (34). These latter discoveries can be verified because we discovered much lower apelin levels in our hypothyroid samples. To the best of our knowledge, this research is the first to examine how apelin interacts with other humoral regulators of fluid-electrolyte homeostasis and thyroid dysfunctional factors. Copeptin and TSH levels, out of the variables examined, were found to be predictors of serum apelin levels, with TSH being a significantly greater determinant. Thyroxine levels, on the other hand, had no effect. Hyponatremia was rare in our study, involving only 5% of patients with hypothyroidism. Serum sodium concentrations, which were correlated with apelin and copeptin levels, were considerably lower in the hypothyroid period in univariate analysis. Although neither apelin nor copeptin levels were independent predictors of serum sodium, and vice versa, serum sodium was not an independent predictor of either apelin or copeptin levels. This could be explained by the fact that our patients' serum sodium levels varied relatively little (5.47 mmol/L) compared to other research looking at the impact of hypertonic saline infusion or water loading test (14). It's interesting to note that the abovementioned changes weren't present in the body fluid, serum, and urine osmolality measurements.

With short-term severe hypothyroidism, hyponatremia was uncommon, and copeptin levels showed no change in the ADH/AVP secretion in our patient population. However, in some sensitive hypothyroid patients, it is possible that a fall in apelin levels brought on by hypothyroidism may be a factor in the development of hyponatremia. In other words, hyponatremia may develop if concurrent ADH/AVP oversecretion also develops, for instance, as a drug side effect due to a high outside temperature or as a result of increased fluid intake. The various comorbidities of hypothyroidism, such as increased atherosclerosis, may also be exacerbated by the lower apelin levels (31, 34).

The literature contains inconsistent information regarding changes in ADH/AVP levels in hypothyroid patients (4, 5, 6, 7). The patient populations' heterogeneity may be to blame for this. Some researchers only investigated young women with long-term hypothyroidism and no concomitant conditions (4).

Elevated serum ADH/AVP levels in chronic hypothyroidism may be caused by cardiac fibrosis and dysfunction stimulating non-osmotic ADH/AVP release (4, 5). As in our model, these modifications may not be present in short-term hypothyroidism. In addition to the various patient demographics, inconsistent results could be the consequence of measurement errors in ADH/AVP levels. Copeptin, however, is a more accurate surrogate marker of ADH/AVP (10). To the best of our knowledge, our research was the first to examine the levels of copeptin in hypothyroidism.

Contrary to the anticipated reciprocal changes in copeptin and apelin plasma concentrations, copeptin and apelin displayed positive relationships with one another and the serum sodium level in our patients. Additionally, copeptin and apelin concentrations throughout multiple regression analyses were significant independent predictors of each other's serum concentrations. Additionally, TSH was a predictor of copeptin and apelin levels, with the connection between the two being particularly robust and accounting for about half of the variance in the latter. fT4 was not a significant variable in these models. These results might imply that in this clinical scenario, the possible changes in these hypothalamic hormones are primarily controlled by hypothyroidism rather than anomalies in the osmotic or volemic state. Abnormalities in the hypothalamic regulation could explain these results; for example, the low apelin level in hypothyroidism could be brought on by increased TRH production, which is prevalent in this condition.

Only the apelin level was associated with NT-proBNP concentrations; neither apelin nor copeptin levels were associated with adrenal cortical hormones. We discovered significantly decreased NT-proBNP concentrations, which is consistent with other reports of atrial natriuretic hormone levels being lower in the hypothyroid state (4, 32). Contrary to past observations in hypothyroid patients or healthy men exposed to a hypertonic solution, aldosterone concentrations did not decrease in our cohort (4, 14). Similar to other investigations, renin activities also increased noticeably as hypothyroidism returned to normal (4, 35). The lack of significant increases in aldosterone concentrations may be partially explained by the 89% boost of NT-proBNP levels observed after the correction of hypothyroidism. The simultaneous rise in ANH levels (measured as NT-proBNP) during the correction of hypothyroidism may balance the effects of increased renin activity and potassium concentrations on aldosterone secretion. Additionally, due to its hemodynamic effects, the rise in apelin levels after hypothyroidism was corrected may be a factor in the elevation of NT-proBNP.

Knowing how apelin secretion is regulated is essential because it affects a variety of disorders linked to hypoxia, including those connected to obesity, diabetes, cancer, heart failure, and increased cardiac output. Additionally, this peptide can be used as a diagnostic for heart conditions and a defense against apoptosis (31).

SUMMARY

The main benefit of our study is the evaluation of numerous possibly associated humoral parameters in a homogeneous patient population free of comorbidities that might have affected the results. We used various statistical techniques to try to understand the potential pathomechanisms underlying our fundamental observations.

However, due to the inherent limits of this approach, it is impossible to explain numerous findings reliably. Our study's requirement for a normal control population is another drawback. Although it would have been challenging to match controls across various characteristics beyond the more common anthropometric ones, our study's self-control pattern allowed us to generate more or less uniform experimental settings.

Additionally, our population had a limited number of participants. However, several of our initial findings and connections had statistically highly significant results.

APELIN-13 AS A POTENTIAL BIOMARKER IN CRITICAL ILLNESS

INTRODUCTION

Patients who require critical care have a high and partially unpredictable fatality rate. Complex prognostic ratings have been developed to enhance outcome prediction. However, despite significant efforts, the prognostic biomarkers' prediction ability is subpar. Finding new biomarkers would be crucial for bettering the prognosis function as well as for a deeper comprehension of the pathomechanisms of critical illnesses (36, 37).

Cortisol and arginine vasopressin (AVP) have been extensively researched in the literature for their predictive functions.

Neuro-hormonal reactions, including increased cortisol levels, greatly influence stress. Due to the hypothalamus-pituitary-adrenocortical (HPA) axis' early activation and impaired cortisol metabolism, free cortisol levels are frequently high in critically ill patients (38, 39). In a diverse group of critically sick patients admitted to the intensive care unit (ICU), we previously showed that free cortisol is an independent predictor of 30-day mortality (40). Free cortisol has also been noted to be a reliable indicator of the inflammatory response in septic shock and its predictive usefulness (41).

The posterior pituitary releases arginine vasopressin, also known as the anti-diuretic hormone, in response to different stressors, including hypovolemia, hypoxia, acidosis, and severe infections. In healthy and seriously ill patients, the copeptin level is a valid surrogate marker for the AVP effect (42). Patients admitted to the ICU have high levels of copeptin, such as septic patients (42, 43, 44). Copeptin also functions as a prognostic indicator; during admission, the serum levels of copeptin are higher in non-survivors (45, 46), and it is closely associated with severity scores (SAPS II and APACHE II) (47). The survival rate is independently predicted by copeptin in septic shock (42, 48) and acute heart failure (49).

AVP activity on the distal convoluted and collecting tubules of the kidney is blocked by the 36-aminoacid peptide hormone called apelin (50). Apelin co-localizes with AVP in magnocellular neurons and is especially abundant in the supraoptic and paraventricular nuclei (51, 52). Its receptor was found in the pituitary gland, cerebral cortex, hypothalamus, and hippocampus (51). Moreover, peripheral tissues possess the apelinergic system: within the heart (53), gastrointestinal tract, skeletal muscle, liver, ovary, kidney, adipose tissue, lung, and endothelial cells (54).

The effects of apelin on HPA function in animal models have been mediated by corticotropin-releasing hormone (CRH) and AVP-dependent pathways (55). Moreover, apelin controls cardiovascular homeostasis, which is crucial for controlling blood pressure, raising cardiac output, and providing cardioprotection from oxidative stress (31, 53, 54). Furthermore, apelin is a renin-angiotensin system-mediated endothelium-dependent vasodilator (1, 50). Besides playing a crucial part in maintaining cardiovascular homeostasis, apelin also functions in glucose metabolism, fluid homeostasis, and other crucial physiological processes (31, 50).

The important apelin isoform in human plasma has been identified as apelin-13 (56).

As a key regulator of the hormonal stress response, CRH promotes the release of both ACTH and AVP (57). Moreover, AVP, which functions as a second "releasing factor" for ACTH and CRH, is expressed by the parvocellular CRH neurons (58). Because it does not correlate well with values seen in the hypothalamic-hypophysial portal plasma, CRH is less frequently tested in peripheral blood samples than ACTH. The expression of CRH is increased by lipopolysaccharide (LPS) in the gut, immunological cells, and the brain (59). To the best of our knowledge, there is no information available regarding the serum CRH level in critically ill patients at admission.

OBJECTIVES

The current study examined a more intricate interaction between the hypothalamus and adrenocortical systems in a diverse group of patients with severe illnesses. Based on the abovementioned information, the hormones apelin-13, copeptin, CRH, free cortisol, and aldosterone were chosen for examination as biomarkers.

MATERIALS AND METHODS

STUDY DESIGN

Patients who were severely ill and were admitted to the University of Pécs Clinical Center's Intensive Care Unit, Emergency Department, or First Department of Medicine were the subjects of our prospective cohort study. The recruitment process was finished between May 2019 and June 2020, as well as June and October 2012. Vital signs, routine laboratory results, and clinical status were all noted. After a thorough examination of the patients' therapies, blood samples that the administration of glucocorticoids had altered were excluded from the future analysis. The SAPS II and APACHE II scoring techniques were used to rate the severity of the condition.

We were given permission to carry out our study in compliance with the 2003 Declaration of Helsinki ethical norms by the University of Pécs Regional Research Ethics Council. Before participating in the study, participants or their parents, legal guardians, or next of kin submitted written informed permission.

PATIENTS

It was a mixed population of patients with medical emergencies; no COVID-19, surgical, or trauma patients were present. Eighteen patients required complete cardiopulmonary resuscitation, while eight patients had been defibrillated prior to admission. Those who passed away within six hours of registering or in circumstances where it was impossible to obtain informed consent were not enrolled. None of the patients received etomidate, ketoconazole, or other medicines modifying steroid metabolism.

DETERMINATION OF ROUTINE LABORATORY TESTS AND NEUROHORMONAL MEDIATORS

The chemistry panel and fully automated blood picture tests were performed at the time of admission using the standard laboratory diagnostic kits and automated equipment of the Department of Laboratory Medicine, University of Pécs (accreditation number: NAH-1-1553/2016).

Also, at the time of admission, blood samples were taken to measure the levels of free cortisol, apelin-13, copeptin, CRH, and aldosterone. They were collected within a Vacutainer (Becton Dickinson, Hungary Kft., Környe, Hungary) plastic tubes without anticoagulants. After centrifuging the obtained blood samples at 2200 x g for 10 minutes, the serum was split into aliquots in Eppendorf tubes and stored at -80°C.

The sample preparation and measurements for free cortisol analysis were done using high-performance liquid chromatography along with high-resolution ESI-TOF mass spectrometry in accordance with the verified method provided by Montsko et al. (60).

Serum apelin-13, copeptin, and CRH levels were measured with the ELISA method using Human Apelin-13 ELISA kit (Catalog No.: abx252028, Abbexa Ltd., UK; intra-assay: CV<10%, inter-assay: CV<10%), Human Copeptin (CT-proAVP) ELISA kit (Catalog No.: abx252269, Abbexa Ltd., UK; intra-assay: CV<10%, inter-assay: CV<10%), and Human Corticotropin-Releasing Hormone (CRH) ELISA Kit (Catalog No.: MBS264947, MyBioSource; intra-assay: CV≤8%, inter-assay: CV≤12%) according to the manufacturer's instructions on a BioTek Synergy HT plate reader at 450 nm. Serum aldosterone was measured using the radioimmunoassay method (Ref: IM1664, RIA-mat 280, Stratec).

STATISTICAL ANALYSIS

SPSS 22.0 was used to conduct the statistical analysis. In parameters where the distribution is normal, data are displayed as the mean and standard deviation (SD), whereas regarding non-normal distribution, they are median and interquartile. To determine if the data were normally distributed, the Shapiro-Wilk test was carried out. In order to determine the correlation between the parameters, the Spearman correlation was used. To compare the groupings, Mann-Whitney U tests were utilized. Using the backward selection method, binary logistic regression analyses were carried out, where a p-value of <0.1 was significant. A p-value of 0.05 was deemed significant in all analyses other than repeated logistic regression analyses; a p-value of 0.1 was deemed significant when using linear methods with backward selection. The direction and strength of the association are reflected in the beta value. Cox regression analysis with backward selection was used to describe survival time; a p-value of <0.1 was significant.

RESULTS

For the trial, a total of 124 participants were enrolled. Patients were a diverse group of people who needed critical care.

Table 6 displays the key characteristics of the patient population.

Table 6. Patients' main characteristics.

Age (years, median, interquartile)	70.0 (59.3-78.0)
Gender (male/female)	64/60
30-day mortality rate	43 of 124
	34.7%
Mechanical ventilation	46.0%
Catecholamine treatment	58.1%
Acute hemodialysis	26.6%
APACHE II score (median, interquartiles)	22.0 (17.0-28.8)
SAPS II score (median, interquartiles)	40.0 (32.0-59.8)
Diagnosis	
Sepsis	30
Heart failure	21
Pulmonary embolism	8
Acute myocardial infarction	8
Primary respiratory failure	20
Critical arrhythmias	10
Other	27

Table 7 shows the SAPS II score and hormonal parameters based on 30-day survival. The free cortisol, CRH, and copeptin levels were significantly above the normal range in both surviving and non-surviving patients with critical diseases (data on the apelin-13 normal range are not available). The median values of sodium, potassium, carbamide, and creatinine were all within the normal range.

Table 7. SAPS II score and hormonal parameters as median (Q1-Q3), according to 30-day survival in the total population.

	Total population	*Normal range*	*Survived on day 30 (n=81; 65.3%)*	*Deceased within 30 days (n=43; 34.7%)*
SAPS II score*	40.0 (32.0-59.8)	Not applicable	**36.0 (23.5-46.0)**	**60.0 (42.0-70.0)**
Free cortisol (nmol/L)	35.4 (9.5-126.3)	1.0-8.0	**25.2 (5.3-89.3)**	**65.3 (29-199.1)**
***Copeptin (pg/mL)**	696.7 (459.0-1106.3)	4.0-52.0	**642.2 (416.4-1174.6)**	**765.4 (568.2-1055.1)**
Apelin-13 (pg/mL)	2023.5 (704.1-3320.4)	Not applicable	2477.44 (800.4-3531.1)	1160.7 (616.6-2966.8)
CRH (pg/mL)	176.3 (80.1-355.6)	3.5-11.4	204.8 (85.2-356.8)	105.2 (75.2-322.1)
Aldosterone (pg/mL)	162.2 (79.3-354.4)	67.0-335.0	155.8 (75.6-296.4)	212.4 (92.5-419.3)

* p-value is between 0.05 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

In Table 8, the median and interquartile ranges of the examined hormonal markers are each shown in relation to the primary admissions diagnosis.

Table 8. The concentrations of hormonal parameters as median (Q1-Q3) are differentiated by the underlying reasons for admission.

	Free cortisol (nmol/L)	**Apelin-13 (pg/mL)**	**CRH (pg/mL)**	**Aldosterone (pg/mL)**	**Copeptin (pg/mL)**
Sepsis (n=30)	116.75 (25.75-222.12)	2869.43 (1155.03-3766.26)	376.28 (106.98-801.45)	127.46 (69.71-395.10)	1565.66 (693.33-3379.01)
Acute heart failure (n=21)	31.70 (5.11-150.10)	3107.15 (1253.58-5180.45)	235.16 (84.65-287.13)	197.49 (112.87-412.84)	880.40 (486.90-1478.55)
Pulmonary embolism (n=8)	4.43 (3.09-8.35)	3036.67 (2503.16-3504.87)	336.18 (208.55-818.56)	117.09 (61.39-230.91)	404.96 (297.36-442.83)
Acute myocardial infarction (n=8)	64.16 (4.20-116.68)	3434.01 (2824.88-5815.75)	318.31 (183.64-805.57)	173.15 (104.99-801.86)	663.94 (273.32-1632.11)
Primary respiratory failure (n=20)	30.71 (12.15-71.65)	648.26 (489.48-2517.09)	89.07 (57.63-230.50)	227.01 (107.13-349.62)	651.76 (508.78-732.72)
Critical arrhythmias (n=10)	25.55 (4.21-93.48)	2269.33 (823.07-3677.17)	158.31 (121.19-299.00)	113.94 (64.57-204.24)	830.66 (439.90-1384.53)
Other (n=27)	35.90 (20.80-67.30)	732.04 (511.79-1741.64)	83.47 (70.45-203.94)	211.94 (66.56-385.67)	513.14 (449.10-745.46)

Table 9 illustrates multiple correlations between hormonal levels and 30-day mortality, severity score, and clinical characteristics.

At admission, hypotension affected 55 patients (about 44%). Hypotension was significantly correlated with free cortisol (r = 0.328, p = 0.001), copeptin (r = 0.226, p = 0.012), aldosterone (r = 0.221, p = 0.014), CRH (r = 0.274, p = 0.002), and SAPS II (r = 0.291, p = 0.001) but not apelin-13 or 30-day mortality.

Table 9. Correlations of hormonal and severity parameters.

	Free cortisol	Copeptin	Apelin-13	Aldosterone	CRH	SAPS II	30-day mortality
Free cortisol		**0.217***	-0.105	**0.359*****	0.098	**0.480*****	**0.280****
Copeptin	**0.217***		**0.214***	0.060	**0.251****	0.106	**0.178***
Apelin-13	-0.105	**0.214***		0.006	**0.685*****	**-0.231****	-0.173
Aldosterone	**0.359*****	0.060	0.006		0.028	**0.197***	0.101
CRH	0.098	**0.251****	**0.685*****	0.028		-0.079	-0.124
SAPS II	**0.480*****	0.106	**-0.231****	**0.197***	-0.079		**0.510*****
30-day mortality	**0.280****	**0.178***	-0.173	0.101	-0.124	**0.510*****	

* p-value is between 0.05 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

CRH, SAPS II, serum sodium, potassium, age, and the presence of kidney damage were independent predictors of serum apelin-13 level as determined by multiple logistic regression analyses in two distinct models (Tables 10/A and 10/B).

Table 10/A. Determinants of serum apelin-13 level by multiple logistic regression analysis.

Dependent variable: Apelin-13	
Investigated parameters	Beta-value
CRH*	**0.405**
SAPS II*	**-0.197**
Sodium*	**-0.152**
Potassium*	**-0.196**
Age*	**0.160**
Free cortisol	-0.025
Copeptin	0.122
Aldosterone	-0.050
Creatinine	0.092
Carbamide	0.190
Sex	0.016
Sepsis	-0.122
R-squared	0.334
Adjusted R-squared	0.262

* p-value is between 0.1 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

Table 10/B. Determinants of serum apelin-13 level by multiple logistic regression analysis.

Dependent variable: Apelin-13	
Investigated parameters	Beta-value
CRH* **	**0.330**
SAPS II*	**-0.281**
Sodium*	**-0.142**
Age*	**0.211**
Kidney injury*	**0.263**
Potassium	-0.157
Free cortisol	-0.060
Copeptin	0.064
Aldosterone	-0.021
Creatinine	0.048
Carbamide	0.034
Sex	0.028
R-squared	0.361
Adjusted R-squared	0.292

* p-value is between 0.1 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

Apelin-13 level was significantly elevated in patients with kidney failure (without vs. with kidney injury: 1438.96 (648.26-3249.84) vs. 2966.79 (1756.83-3835.65), p=0.005). Using the same model, the independent predictors of serum CRH level were apelin-13 level and kidney injury (Table 10/C).

Table 10/C. Determinants of serum CRH level by multiple logistic regression analysis.

Dependent variable: CRH	
Investigated parameters	Beta-value
Apelin-13* **	**0.374**
Kidney injury*	**0.209**
SAPS II	-0.033
Sodium	0.086
Potassium	0.032
Age	0.018
Free cortisol	0.127
Copeptin	0.111
Aldosterone	0.028
Creatinine	-0.187
Carbamide	0.011
Sex	-0.094
R-squared	0.277
Adjusted R-squared	0.199

* p-value is between 0.1 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

Hormone levels below and above the median SAPS II score can be seen in Table 11.

Table 12 shows hormone levels based on the septic state. Free cortisol indicated the clearest difference: septic patients exhibited an almost six-fold rise compared to the non-septic population. Additionally, the copeptin and CRH levels were very high.

Due to the large interindividual variability and small number of septic patients, the difference in the apelin-13 median between the septic and non-septic groups was not statistically significant. Sepsis had an impact on renal function, and there was a substantial increase in carbamide (p = 0.001) and creatinine (p = 0.005) levels.

Table 11. Comparison of medians (Q1-Q3) of hormone levels below and above the median of the SAPS II score.

	Below the median of SAPS II (n=62)	Above the median of SAPS II (n=62)
Free cortisol (nmol/L)* **	**12.67 (3.24-59.73)**	**73.40 (30.50-202.08)**
Copeptin (pg/mL)	662.69 (434.38-1028.10)	714.09 (502.95-1739.63)
Apelin-13 (pg/mL)*	**2877.53 (854.18-3488.88)**	**1261.17 (618.04-3152.83)**
CRH (pg/mL)	204.36 (86.72-316.51)	127.44 (75.49-379.54)
Aldosterone (pg/mL)	131.32 (69.27-282.75)	215.04 (109.69-403.77)

* p-value is between 0.05 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

Table 12. Comparison of medians (Q1-Q3) of SAPS II score and hormone levels between the septic and non-septic groups.

	No sepsis (n=94)	Sepsis (n=30)
SAPS II score*	**39.00 (26.00-58.25)**	**49.00 (36.75-64.50)**
Free cortisol (nmol/L)**	**30.50 (5.96-92.75)**	**171.53 (38.30-276.80)**
Copeptin (pg/mL)***	**649.62 (438.87-908.51)**	**1636.60 (694.24-2934.39)**
Apelin-13 (pg/mL)	1479.02 (645.14-3249.84)	3230.44 (2228.96-4012.91)
CRH (pg/mL)**	**132.19 (74.90-278.58)**	**573.09 (322.09-877.30)**
Aldosterone (pg/mL)	172.64 (81.76-340.91)	128.45 (67.00-543.76)

* p-value is between 0.05 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

SAPS II score and hormonal parameters based on 30-day survival in the non-septic subgroup (N = 94 patients; 75.8% of the overall population) are presented in Table 13.

Table 13. SAPS II score and hormonal parameters as median (Q1-Q3), according to 30-day survival in the non-septic subgroup.

	Survived at day 30 (n=64)	Deceased within 30 days (n=30)
SAPS II score*	**34.5 (23.0-43.25)**	**60.5 (41.25-68.5)**
Free cortisol (nmol/L)*	**23.90 (4.43-71.65)**	**35.92 (20.78-137.75)**
Copeptin (pg/mL)*	**542.43 (414.22-879.73)**	**749.41 (511.79-889.78)**
Apelin-13 (pg/mL)*	**2286.17 (789.73-3330.20)**	**817.64 (574.01-2731.69)**
CRH (pg/mL)*	**201.44 (83.77-316.51)**	**89.08 (73.56-233.23)**
Aldosterone (pg/mL)	158.12 (77.97-297.01)	223.55 (108.41-415.26)

* p-value is between 0.05 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

Among the hormonal measures under investigation, free cortisol and apelin-13 were highly reliable independent predictors of death. In the entire population, free cortisol was the best predictor of mortality, whereas apelin-13 overtook free cortisol in the non-septic subgroup (Table 14).

Figure 4/A–4/F shows the survival function at the mean of apelin-13 and CRH in the entire population and according to the presence of sepsis. Except for the septic patient group, where the apelin-13 level was considerably greater in non-survivors, there was no difference in the survival of those patients whose apelin-13 and CRH levels were below and above the mean. Interestingly, non-septic patients showed the reverse pattern.

Table 14. Multivariate Cox regression analysis of the overall survival according to hormonal parameters.

Investigated parameters	Chi-Square in the whole population	Chi-Square in the non-septic group
Free cortisol	**4.69***	4.08
Apelin-13	**3.33***	**3.20***
Copeptin	0.01	0.70
CRH	0.09	0.02
Aldosterone	0.79	1.23
Number of observations	124	94

* p-value is between 0.1 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001

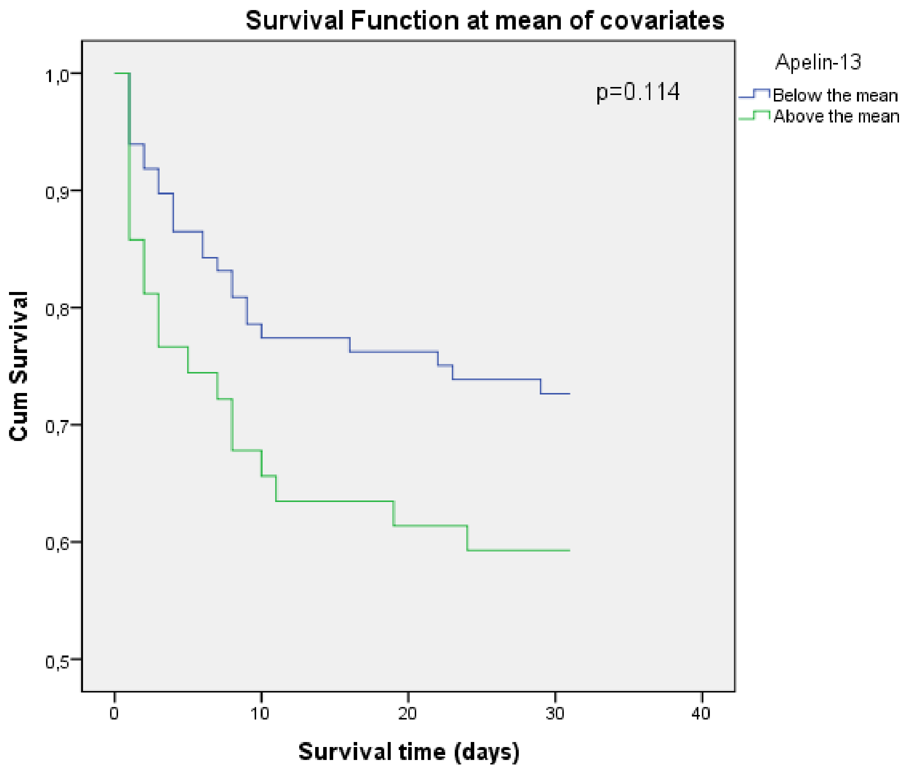

Figure 4/A. Survival function at the mean of apelin-13 in the whole population.

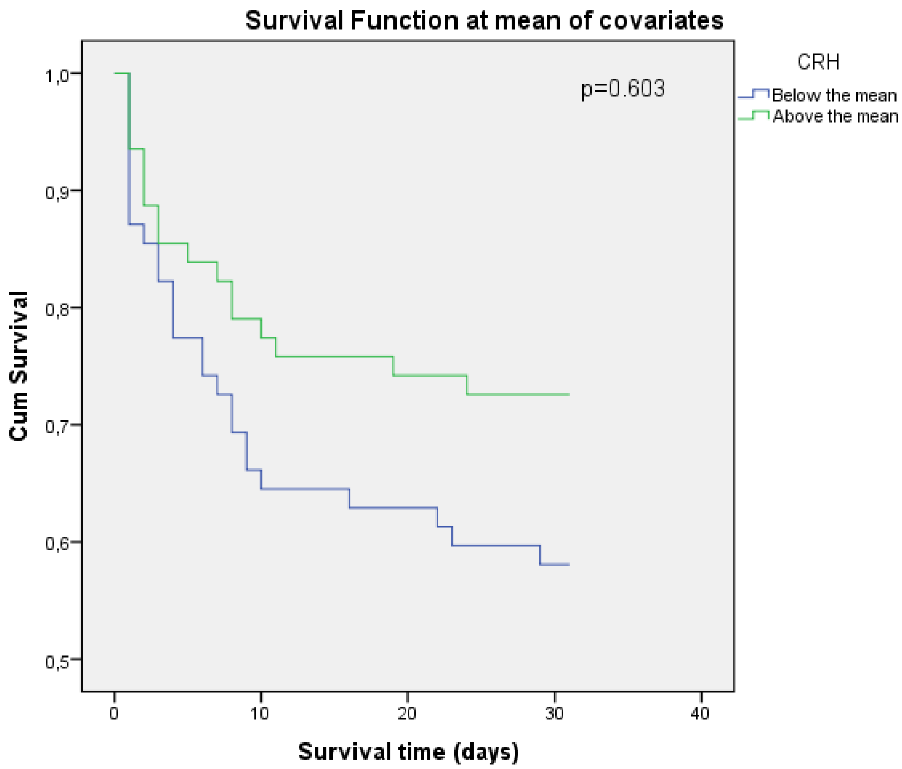

Figure 4/B. Survival function at the mean of CRH in the whole population.

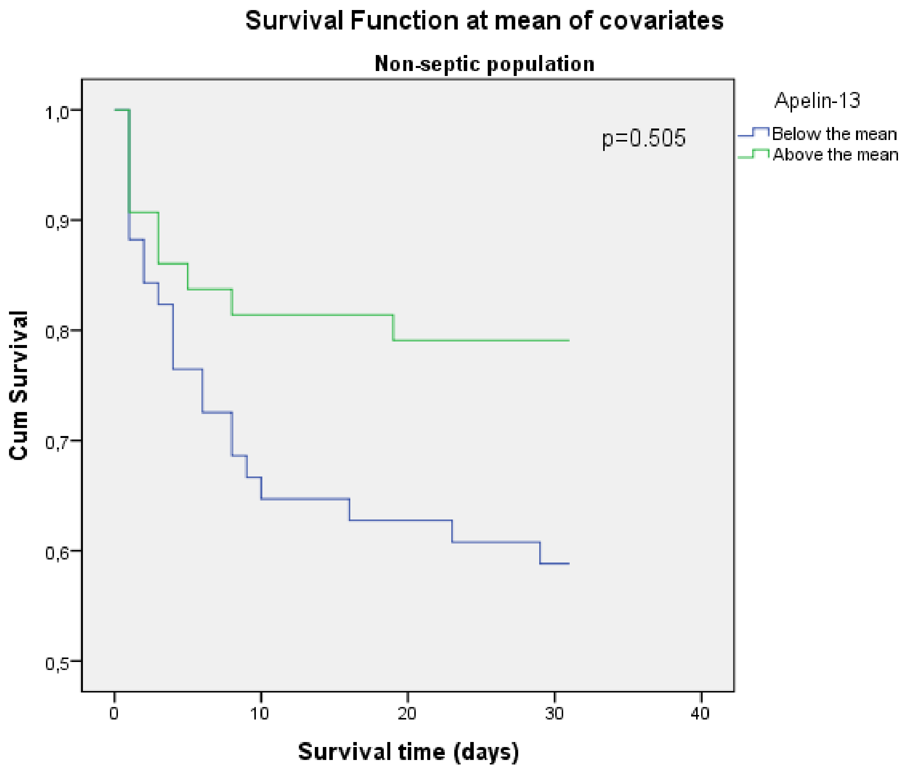

Figure 4/C. Survival function at the mean of apelin-13 in the non-septic population.

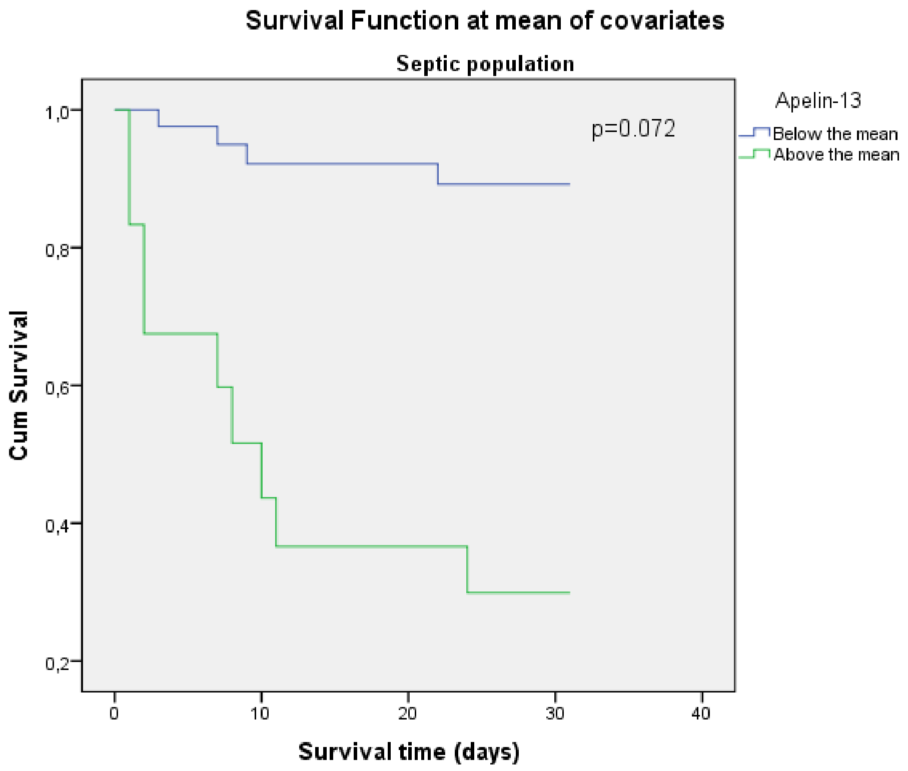

Figure 4/D. Survival function at the mean of apelin-13 in the septic population.

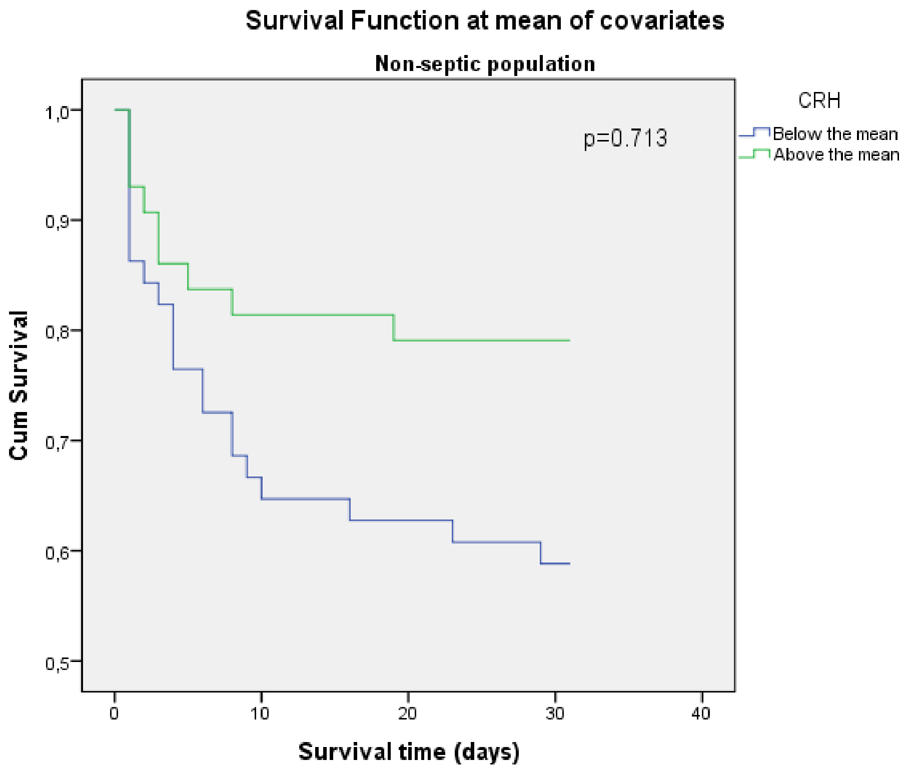

Figure 4/E. Survival function at the mean of CRH in the non-septic population.

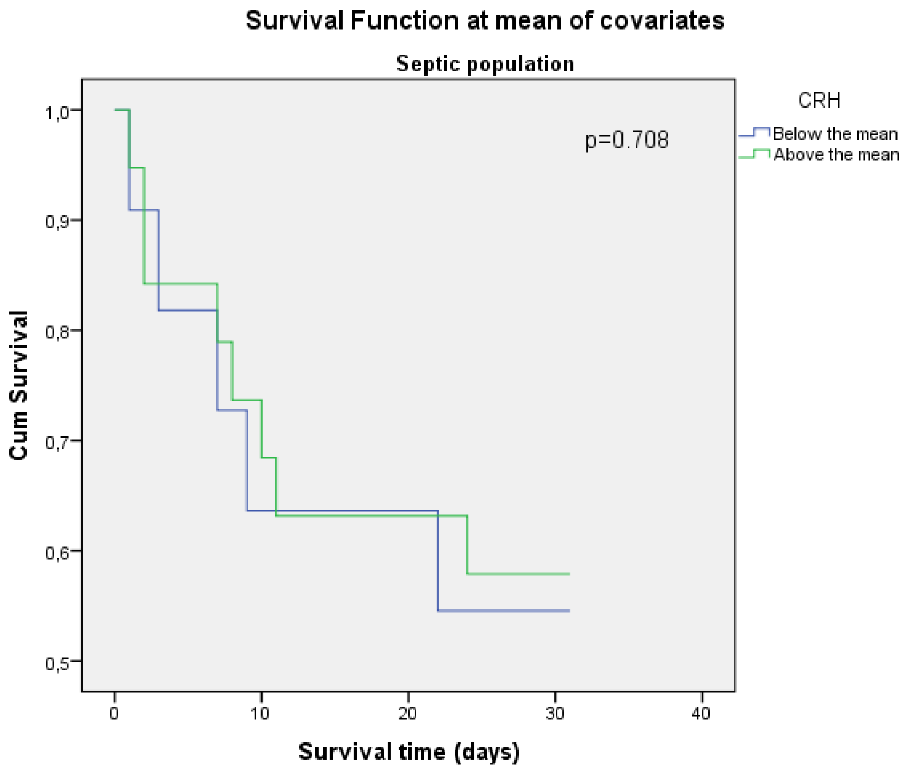

Figure 4/F. Survival function at the mean of CRH in the septic population.

DISCUSSION

Our study investigated numerous hypothalamus and adrenal hormones in a patient population with mixed critical illnesses. Previous articles have shown that non-survivors had significantly higher serum levels of free cortisol and copeptin (40, 45, 46). Apelin-13 displayed a different trend, with a much lower level in more serious instances indicated by scores higher than the median SAPS II score. The subgroups with higher SAPS II severity scores and non-septic non-survivors had significantly lower serum apelin-13 levels. A strong negative correlation between apelin-13 concentration and the SAPS II severity score was also found in univariate and multivariate tests. The independent determinants of survival were discovered using Cox regression analysis. There were several models that included routinely examined laboratory parameters. These models are suitable to assess the significance of the different components depending on the order of elimination. Apart from free cortisol, apelin-13 was an independent factor of survival among the examined humoral markers. Aldosterone, copeptin, and CRH were also dropped out simultaneously. It's noteworthy to note that the survival curves based on the apelin-13 mean exhibited a different trend in patients with septic and non-septic diseases. A greatly higher apelin-13 level was linked to worse survival in the septic subgroup. The Cox regression analysis of the non-septic group revealed a trend toward worse survival in those with lower apelin-13 levels. Therefore, apelin-13 regulation in septic and non-septic critically ill circumstances may differ, and additional research is needed to include a larger proportion of septic patients.

Apelin has been identified as a crucial biomarker for heart failure (61). Additionally, whereas plasma apelin concentrations were lower immediately following myocardial infarction, they did not correspond with the measures of left ventricular function (62). Major adverse cardiovascular events were noticeably more frequent in patients with ST-segment elevation myocardial infarction in the low apelin group compared to the high apelin group (63). To the best of our knowledge, this work is the first to show a negative correlation between serum apelin-13 concentrations and the seriousness of critical disease. Lesur et al. found no evidence of associations between apelin-12, another apelin isoform, and severity or prognosis in critically ill patients presenting systemic inflammatory response syndrome, which is in contrast to our findings (64). The diverse patient groups (septic versus non-septic, for example, following myocardial infarction) and/or the various apelin isoforms under investigation may be the cause of these inconsistent outcomes. In the study by Lesur et al., for instance, despite the numerous concordant, well-demonstrated results demonstrating considerable elevation of copeptin in these patients, even the copeptin was not significantly greater in critically sick patients than in normal volunteers (42, 43, 44, 47, 65, 66).

Under some environmental strain, the apelin system's biological effectiveness is jeopardized. For instance, endogenous apelinergic levels rise early in human sepsis, and some enzymatic breakdown activities may endanger endogenous apelin system reactivity and have a detrimental effect on the outcome (67). Additionally, the short-term exogenous apelin-13 infusion aids in stabilizing cardiorenal functioning in animals suffering from septic shock; however, this capability may be compromised by particular enzymatic systems activated during the initial stages of human sepsis (67).

To the best of our knowledge, this is also the first study to investigate serum CRH levels in a critically ill population. Serum CRH levels were significantly above the reference range in these patients, notably in the septic patients, and positively correlated with apelin-13 and copeptin but oddly not with free cortisol. Additionally, serum CRH has dramatically influenced the levels of apelin-13 in these patients. Additionally, comparable to apelin-13, serum CRH was considerably greater in non-septic patients who survived than in those who passed away within 30 days. Since there are no prior studies on humans, the explanation for the significant correlation between CRH and apelin-13 is purely hypothetical; an acute stress reaction may be to blame for the elevation of both hormones. We still have a lot to learn about how CRH and apelin-13 degrade. It is widely recognized that the reduced degradation process has a role in the increased cortisol levels associated with severe disease (39). It could explain why there is no relationship between free cortisol and CRH. CRH was not a significant parameter in the Cox regression survival analysis, in contrast to apelin-13 levels.

Although the hypothalamus is the primary site of expression for both apelin-13 and CRH, other factors control their serum concentrations. They have tenuous connections with the central nervous system's local effects (31). It is possible to assume that these hormonal systems have more complicated control and stimulating triggers.

It has been shown that early admitted ICU patients respond differently to stress in septic vs. non-septic situations (64).

Our patients in the septic subgroup experienced a more serious illness with a higher SAPS II score. In addition, they had higher levels of the hormones under investigation than non-septic individuals, with the exception of aldosterone; however, the difference in apelin-13 levels was not statistically significant.

Free cortisol and the SAPS II severity score were associated with serum aldosterone, but there were no notable relationships between aldosterone and the other variables.

Numerous attempts have been made to increase critical illness survival and produce a more reliable prognosis score. In multi-organ failure, there are no effective disease-modifying therapies. Three advantages may result from studying the function of apelin-13: a better understanding of the pathophysiological process and the intricate regulation of this hormonal system; the discovery of a prognostic marker that might be less complicated than the prognostic scores currently in use (which contains seventeen parameters); and the identification of a potential therapeutic target.

Evidently, the apelin system is controlled in critical illness, and this control appears to vary between septic and non-septic states. The apelin-13 level in sepsis is abnormally high; patients below the mean apelin-13 level have a greater chance of survival. It is doubtful whether administering exogenous apelin-13 will further elevate apelin-13 in this patient population, where apelin-13 elevation may be a sign of severity. Only apelin-13 remained a significant predictor of survival in the non-septic patient category after multivariate Cox regression analysis. Exogenous apelin-13 injection may enhance the prognosis in non-septic patients with circulatory failure and low apelin-13 levels.

SUMMARY

In more severe cases of the mixed population with a serious illness, serum apelin-13 levels were lower. In terms of apelin-13 levels, it appears that there is a significant difference between the septic and non-septic populations. Both hormone levels were much greater in surviving non-septic patients, and there was a clear positive association between the apelin-13 and CRH concentrations. Apelin-13 and free cortisol were independent predictors of survival in the multivariate Cox regression analysis of the hormonal parameters under investigation, whereas copeptin, CRH, or aldosterone were not.

HUMORAL AND BODY COMPOSITION INVESTIGATIONS IN HYPONATREMIA-RELATED DISORDERS

INTRODUCTION

The most prevalent form of electrolyte imbalance, known as hyponatremia, is defined by a low serum sodium concentration that is typically below 135 mmol/L (68). An estimated 15–20% of all hospitalized patients have this disease. The enlargement of the brain cells brought on by the shift of fluids from the extracellular to the intracellular compartment gives rise to more dramatic clinical symptoms of hyponatremia than a quick decline in serum sodium. The intracranial pressure rises as a result of these modifications. There is a wide range in the degree of symptoms, from total symptom absence to minor symptoms like headache, loss of appetite, nausea, imbalance, and falls to serious symptoms like impaired cognitive abilities, muscle spasms, fractures, osteoporosis, seizures, epilepsy, and coma (69, 70, 71, 72). In addition, it has been shown that mild hyponatremia poses a separate risk for mortality in the ambulatory context (73).

Although the underlying causes of hyponatremia vary, two primary mechanisms — water retention and salt loss — lead to low serum sodium. The circulation volume may be reduced, normal, or elevated depending on the underlying etiology of hyponatremia, leading to hypovolemic, euvolemic, or hypervolemic hyponatremia, respectively (74). The euvolemic and hypervolemic forms usually do not represent diagnostic difficulties. Clarifying the cause of conditions with low plasma osmolarity is even more of a problem (70). The underlying causes of hypovolaemic hyponatremia can be divided into two groups: extrarenal and renal causes. The former include vomiting, diarrhea, fistulas, laxatives, intestinal obstruction, pancreatitis, peritonitis, ascites, increased sweating, trauma, and burns. Diuretic treatments, tubular diseases, the convalescent phase of acute tubular necrosis, various tubular toxins (acetaminophen), the condition after the resolution of obstructive uropathy, chronic kidney diseases (polycystic kidney), mineralocorticoid deficiency and cerebral salt wasting syndrome represent causes belonging to the renal group. Euvolaemic hyponatremia can be caused by SIADH (syndrome of inappropriate ADH secretion), glucocorticoid deficiency, hypothyroidism, and diuretic treatment. Hypervolaemic hyponatremia develops in case of inadequate parenteral fluid therapy or conditions with edema (heart failure, liver cirrhosis, nephrosis syndrome, kidney failure) (75).

Edematous disorders are typically treated with thiazide and loop diuretics, which are the most frequent causes of drug-induced hyponatremia (76).

Although both kinds of diuretics cause natriuresis, there may be differences in how they affect water balance. Thiazides block the Na-Cl cotransporter in the cortical dilating portion of the nephron, the distal convoluted tubule. Thiazides may, therefore, cause renal water retention and impaired urine dilution (77). According to Liamis et al., patients with thiazide-induced hyponatremia exhibit symptoms of SIADH, such as low blood uric acid concentrations and increased fractional excretion of uric acid (78). However, there have been conflicting findings regarding the measurement of plasma AVP in patients with thiazide-induced hyponatremia. Some studies reported elevated AVP concentrations (79, 80), while others did not (81, 82, 83).

While other drugs can cause hyponatremia through one of three possible mechanisms: (I) central increase in ADH secretion; (II) potentiation of the effects of endogenous ADH; or (III) lowering of the threshold for ADH secretion. Most psychiatric medications thought to cause hyponatremia are supposed to do so by causing SIADH. Several antidepressant drugs (ADDs, including SSRIs, MAOIs, and tricyclic antidepressants (TCAs)), antipsychotic drugs (APDs), and antiepileptic drugs (AEDs) are associated with a risk of causing hyponatremia (76, 84, 85).

It would be a huge step forward in diagnosing these diseases if we had a reliable laboratory parameter for ADH secretion. In the future, the routine availability of copeptin determination may create an opportunity for this. It is well known in the medical community that, for example, small-cell lung cancer often produces ADH (86). However, drug-induced hyponatremia often goes unrecognized, even in the most severe cases. Drugs that often cause hyponatremia and the mechanism of hyponatremia are summarized in Table 15. Several other drugs have also been described as causing low sodium levels, and these are listed as rare causes in Table 16. (76).

Table 15. Drugs that often cause hyponatremia and the pathomechanism of hyponatremia.

Agents affecting salt and water balance	Stimulators of hypothalamic ADH secretion	Agents that sensitize the effect of ADH	Agents that reduce the threshold of ADH secretion
Diuretics Thiazide Indapamide Amiloride Loop diuretics	*Antiepileptic drugs* Carbamazepine Oxcarbazepine Valproic acid	*Antiepileptic drugs* Carbamazepine Lamotrigine	*Antiepileptic drugs* Carbamazepine
	Antidepressants Tricyclic antidepressants (TCAs) Selective serotonin reuptake inhibitors (SSRIs) Monoamine oxidase inhibitors (MAOIs)	*Non-steroidal anti-inflammatory drugs*	*Antidepressants* Venlafaxine
	Chemotherapy drugs Vinca alkaloids Platinum-based drugs Alkylating agents Others Methotrexate Interferon	*Chemotherapy drugs* Alkylating agents Cyclophosphamide	
	Antipsychotics Butyrophenone Phenothiazine		
	Opiates		

Table 16. Rare causes of drug-induced hyponatremia.

Antihypertensive drugs
ACE inhibitors
Amlodipine
Proton pump inhibitors
Antibiotics
Ciprofloxacin
Trimethoprim-sulfamethoxazole
Antiarrhythmic medications
Amiodarone
Propafenone
Immunoglobulin therapy
Theophylline
Bromocriptine
Other antidepressants
Bupropion
Duloxetine

OBJECTIVES

This study aims to analyze and compare hyponatremic patients' laboratory and body composition data at the University of Pécs, Medical School, 1st Department of Medicine, Division of Endocrinology and Metabolism, between February 2018 and January 2019.

MATERIALS AND METHODS

STUDY DESIGN

We conducted a prospective cohort study on hyponatremic patients at the University of Pécs, Medical School, 1st Department of Medicine, Division of Endocrinology and Metabolism. The recruitment and control measurements were completed between February 2018 and March 2019. Clinical status, laboratory parameters, and vital signs were assessed. Patients with serum sodium levels below 136 mmol/L were confirmed as eligible for the study.

We received approval from the University of Pécs Regional Research Ethics Council to conduct our study in accordance with the Declaration of Helsinki ethical principles from 2003. Participants or the participants' parents, legal guardians, or next of kin provided written informed consent before participating in the study.

PATIENTS

Nineteen hyponatremic patients, 4 men and 15 women, were examined within 32 hours of detection in the Accident and Emergency Department, whose average age was 79 (64-83). Body composition and humoral parameters regulating salt-water balance were also measured in the case of 11 patients during a six-week control examination.

DETERMINATION OF BODY COMPOSITION, ROUTINE LABORATORY TESTS, AND NEUROHORMONAL MEDIATORS

For additional biochemical investigation, venous blood samples were taken right after the admission. After that, body weight was measured to the nearest kilogram and height to the nearest centimeter. Then, we measured the waist circumference in the horizontal plane halfway between the lowest rib and the iliac crest and the hip circumference over the most prominent area of the buttocks. To eliminate inter-individual variations, the same researcher took all the anthropometric measurements.

Body composition was evaluated using a bioelectrical impedance analysis (BIA) device (Bodystat Quadscan 4000, Bodystat Ltd., P.O. Box 50, IM99 1DQ Douglas, Isle of Man, United Kingdom). Before the measurement, patients were laid out in the supine position for at least five minutes.

All participants wore light clothing and removed earrings, rings, bracelets, and any other metal which could influence the measurement results. Every measurement took each participant about 30 seconds.

Only patients who complied with the BIA protocol, which calls for abstaining from alcohol for 48 hours, refraining from strenuous activity for 12 hours, and fasting for at least four hours before the test, were accepted. Patients who had implanted electronic devices, such as pacemakers for the heart, were disqualified.

Using the standard laboratory diagnostic kits and automated equipment of the Department of Laboratory Medicine (accreditation number: NAH-1-1553/2016), the chemistry panel, hormonal parameters, and completely automated blood picture tests were determined at the time of admission. Serum aldosterone was measured using the radioimmunoassay method (Ref: IM1664, RIA-mat 280, Stratec).

STATISTICAL ANALYSIS

The statistical analysis was carried out using SPSS 22.0. Data are shown as mean SD in parameters with normal distribution, while median and interquartile values are in the non-normal distribution. The Shapiro-Wilk test was used to check for normal distribution. Spearman correlation was utilized to ascertain the relationship between the parameters in the whole population. Paired sample t-test and Wilcoxon test were used to compare the patients' parameters between the two states in normal and non-normal distribution, respectively.

RESULTS

The average admission sodium level was 113.4 ± 1.6 mmol/L, and the lowest was 99 mmol/L. Our patients were all admitted to the ward with severe hyponatremia (serum sodium level below 125 mmol/L) after detection in the Accident and Emergency Department (Figure 5).

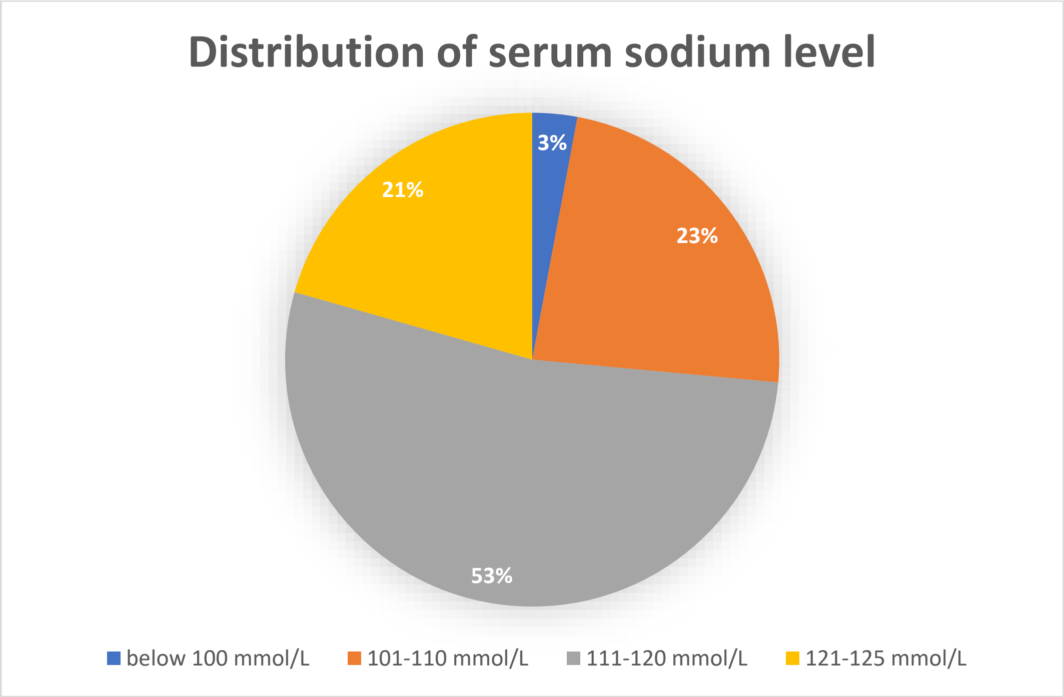

Figure 5. Distribution of serum sodium level at admission.

Drug-induced hyponatremia was probable in 17/19 patients, of which 14/19 cases were a side effect of thiazide or thiazide-like diuretics (Figure 6).

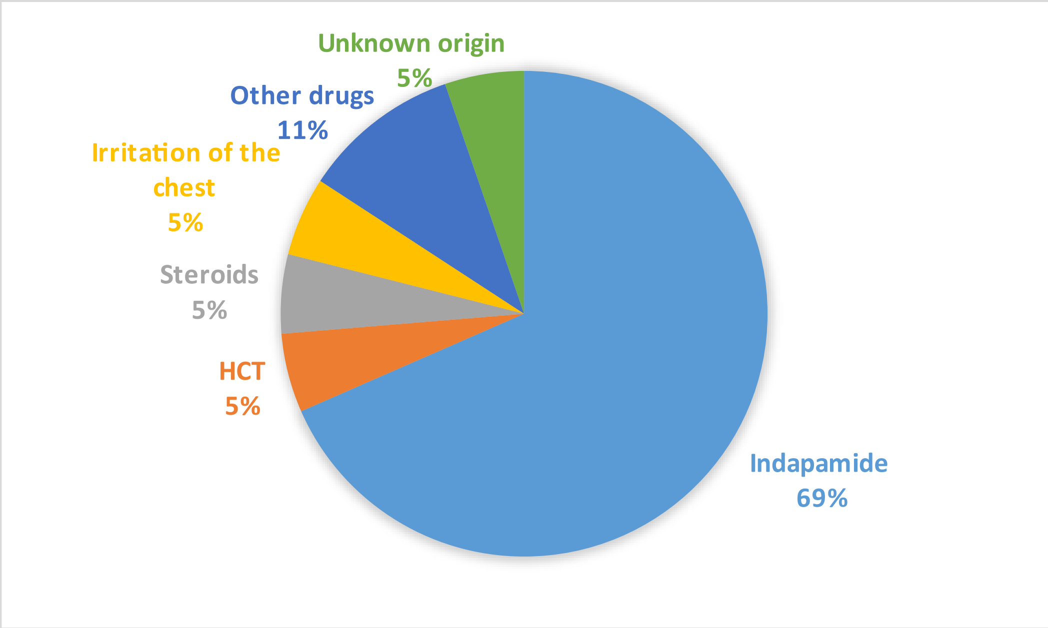

Figure 6. The presumed causes of hyponatremia in our population (N=19).

In four of these cases, urine Na was low (<20 mmol/L), in the others, it was high (at least 30 mmol/L) (Figure 7).

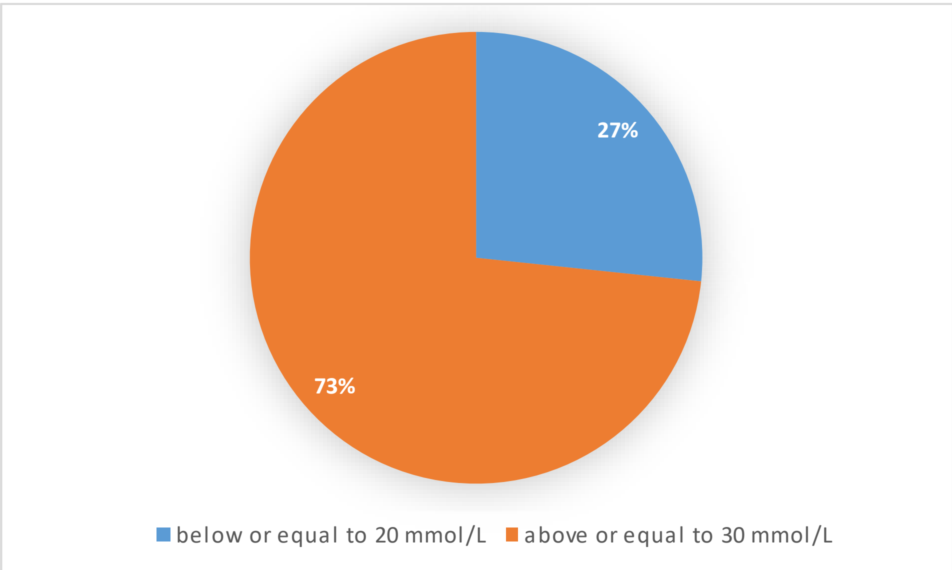

Figure 7. Distribution of urine sodium

Overcorrection (serum Na increase >10 mmol/L/24 h) occurred in more than a quarter of the patients (5/19). In 73.7% of patients (14 patients), the hyponatremic episode did not occur only once but was repeated (median: 4.5, max. 9) (Figure 8).

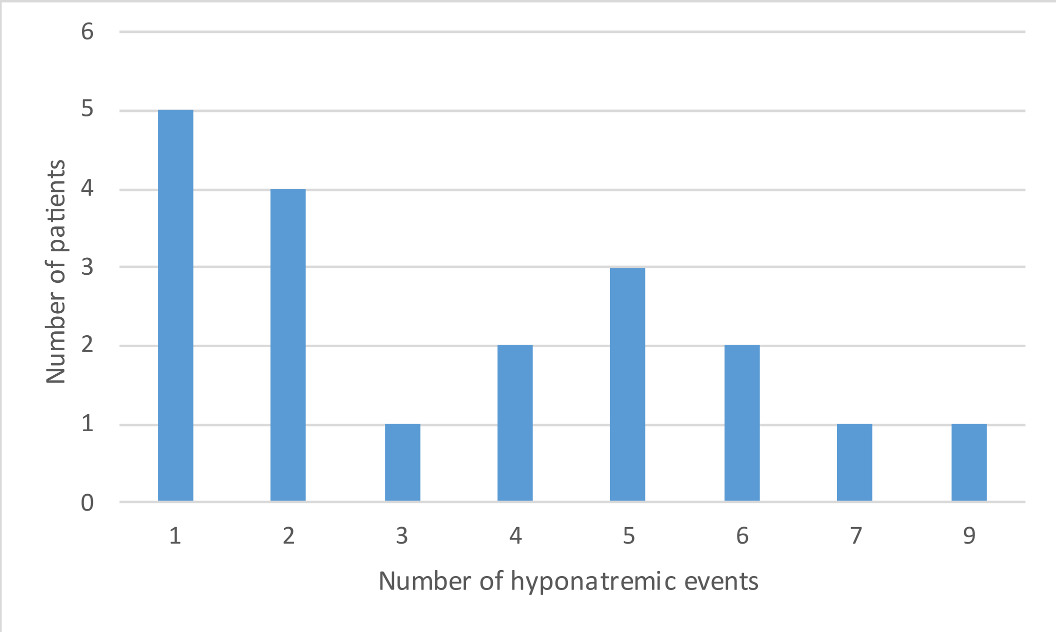

Figure 8. Frequency of hyponatremic episodes.

During the control examinations, the patients' total body water, intracellular water, serum potassium, serum chloride, serum osmolarity, and uric acid increased significantly, and only one patient had hyponatremia. Despite the (relative) improvement of the general condition, there was a significant decrease in eGFR. The intracellular water space was significantly larger during the control, and the serum cortisol level decreased significantly (Table 17).

Table 17. Examination of changes in water spaces and main laboratory parameters characterizing salt-water balance in hyponatremia and six weeks after treatment.

Parameters	Hyponatremic phase	Control phase	p-value
TBW (L)	**36.2 (31.5-41.3)**	**38.0 (33.4-43.8)**	0.03
ECW (L)	15.9 ± 3.2	16.1 ± 3.1	0.50
ICW (L)	**21.0 (17.3-26.6)**	**21.3 (19.0-26.8)**	0.045
Serum sodium (mmol/L)	**116.0 (109.8-120.0)**	**139.0 (138.0-141.8)**	0.001>
Serum potassium (mmol/L)	**3.9 ± 0.9**	**4.7 ± 0.4**	0.004
Serum chloride (mmol/L)	**83.2 ± 8.0**	**100.5 ± 3.6**	0.001>
Serum osmolarity (mOsmol/kg)	**249.0 (236.0-268.0)**	**283.0 (276.0-292.0)**	0.001
Carbamide (mmol/L)	4.6 (3.1-6.6)	4.9 (3.8-7.0)	0.833
Creatinine (μmol/L)	67.5 (51.8-84.3)	74.5 (67.0-82.8)	0.095
eGFR (mL/min/1.73m^2)	**73.7 ± 16.9**	**67.0 ± 14.1**	0.046
Uric acid (μmol/L)	**180.0 (125.5-266.0)**	**278.5 (205.8-327.3)**	0.024
ACTH (pg/mL)	16.0 (10.0-21.2)	14.6 (10.8-22.8)	0.758
Cortisol (nmol/L)	**544.0 (419.7-683.6)**	**369.0 (266.1-464.2)**	0.001
Aldosterone (pg/mL)	138.4 (101.2-181.9)	152.6 (85.0-209.0)	0.601
Renin (ng/mL/h)	1.2 (0.3-4.2)	0.7 (0.3-1.6)	0.411
Urine sodium (mmol/L)	45.0 (18.8-61.0)	50.0 (36.5-105.0)	0.462

The whole population was included to determine the relationship between the laboratory parameters.

Significant correlations are shown in Table 18 between sodium and potassium, chloride, osmolarity, creatinine, eGFR, and uric acid; and between serum potassium and serum chloride, osmolarity, carbamide, creatinine, eGFR, and uric acid.

Table 18. Correlation of sodium and potassium with the most important laboratory parameters and the ratio of water spaces. Significant values are labeled as bold.

	Serum Na	p-value	Serum K	p-value
Serum sodium (mmol/L)	R: 1		**R: 0.481**	0.001>
Serum potassium (mmol/L)	**R: 0.481**	0.001>	R: 1	
Serum chloride (mmol/L)	**R: 0.917**	0.001>	**R: 0.451**	0.003
Serum osmolarity (mOsmol/kg)	**R: 0.889**	0.001>	**R: 0.410**	0.008
Carbamide (mmol/L)	R: 0.292	0.061	**R: 0.434**	0.004
Creatinine (μmol/L)	**R: 0.348**	0.010	**R: 0.382**	0.004
eGFR (mL/min/1.73m^2)	**R: -0.335**	0.015	**R: -0.420**	0.002
Uric acid (μmol/L)	**R: 0.545**	0.001>	**R: 0.351**	0.010
ICW/ECW ratio	R: -0.049	0.736	R: 0.063	0.662

Investigating hormones in more detail, we found significant correlations between renin and aldosterone, urine sodium; aldosterone and uric acid, urine sodium; ACTH and creatinine, eGFR; cortisol and serum sodium, potassium, chloride, osmolarity (Table 19).

Table 19. Correlations of renin, aldosterone, ACTH, and cortisol with important parameters characterizing salt-water balance. Significant values are labeled as bold.

Parameters	Renin	Aldosterone	ACTH	Cortisol
Renin (ng/mL/h)	R: 1	**R: 0.357 (p=0.008)**	R: 0.053 (p=0.702)	R: 0.082 (p=0.558)
Aldosterone (pg/mL)	**R: 0.357 (p=0.008)**	R: 1	R: -0.050 (p=0.718)	R: 0.241 (p=0.079)
ACTH (pg/mL)	R: 0.053 (p=0.702)	R: -0.050 (p=0.718)	R: 1	R 0.002 (p=0.991)
Cortisol (nmol/L)	R: 0.082 (p=0.558)	R: 0.241 (p=0.079)	R 0.002 (p=0.991)	R: 1
Serum sodium (mmol/L)	R: -0.159 (p=0.250)	R: 0.055 (p=0.695)	R: 0.019 (p=0.892)	**R: -0.428 (p=0.001)**
Serum potassium (mmol/L)	R: -0.078 (p=0.575)	R: -0.092 (p=0.509)	R: 0.255 (p=0.062)	**R: -0.313 (p=0.021)**
Serum chloride (mmol/L)	R: -0.090 (p=0.581)	R: 0.095 (p=0.559)	R: 0.089 (p=0.586)	**R: -0.529 (p<0.001)**
Serum osmolarity (mOsmol/kg)	R: -0.120 (p=0.455)	R: -0.007 (p=0.964)	R: 0.121 (p=0.449)	**R: -0.619 (p<0.001)**
Carbamide (mmol/L)	R: 0.098 (p=0.538)	R: 0.233 (p=0.137)	R: 0.207 (p=0.188)	R: -0.266 (p=0.089)

Creatinine (μmol/L)	R: 0.013 (p=0.928)	R: 0.193 (p=0.162)	**R: 0.332 (p=0.014)**	R: -0.104 (p=0.454)
eGFR (mL/min/1.73m^2)	R: 0.073 (p=0.609)	R: -0.195 (p=0.165)	**R: -0.352 (p=0.011)**	R: 0.219 (p=0.119)
Uric acid (μmol/L)	R: 0.006 (p=0.965)	**R: 0.296 (p=0.032)**	R: 0.139 (p=0.322)	R: -0.263 (p=0.057)
ICW/ECW ratio	R: -0.106 (p=0.463)	R: -0.179 (p=0.213)	R: 0.195 (p=0.174)	R: -0.177 (p=0.218)
Urine sodium (mmol/L)	**R: -0.475 (p=0.008)**	**R: -0.520 (p=0.003)**	R: 0.039 (p=0.839)	R: 0.094 (p=0.620)

DISCUSSION

We can draw several conclusions after processing the two-year patient material hospitalized for drug-, primarily diuretic-induced hyponatremia.

All of the examined patients were admitted with a severe degree of hyponatremia (<125 mmol/L), similar to the results previously described by our research group, where this value was 88%. The phenomenon of hyponatremia can be considered much more common; the cases are only sometimes recognized, and only in the most serious situations are they admitted to the hospital.

Over 70% of patients had a hyponatremic event more than once during the last ten years of study. In our previous investigations, this number was 77%.

Spring-summer seasonality was also typical in our studied population but less markedly than before. A 10-year prospective study in Sweden examined 1282 patients admitted for hyponatremia. A significant correlation was found between outdoor temperature and the development of drug-induced severe hyponatremia (87).

Unfortunately, overcorrection of hyponatremia occurred quite often, representing 35% of cases. In one case, this resulted in central pontine myelinolysis, with a permanent inability to swallow, which also required PEG implantation. He was admitted to the department due to severe hyponatremia (serum Na^+: 102 mmol/L), hypokalemia (serum K^+: 3.15 mmol/L), and, based on this, difficulty in speaking and slowed psychomotor, which was presumably due to the effect of the diuretic treatment (indapamide). After admission, indapamide was discontinued, ion correction was started, and fluid restriction was applied, as a result of which the patient's general condition improved, and his ion balance was settled. On the fifth day after discharge, the patient presented to the emergency department due to a speech disorder and difficulty walking. The brain MRI confirmed central pontine myelinolysis, the background of which is likely to be the prolonged reflex response to ion correction characteristic of the individual. The fact that the chloride level was also reduced at the initial admission can be explained by the fact that the thiazide-sensitive Na+-Cl- cotransporter is responsible for transporting chloride (88, 89).

Regarding co-morbidities - as expected - hypertension was the most common (31 cases), in the treatment of which indapamide, which causes hyponatremia, is often present.

In addition, medical conditions such as CAD (8 cases) and heart failure (5 cases) may contribute to increased diuretic sensitivity, as can certain other medications taken. Analyzing the most common comorbidities, Nadal and his colleagues found that cardiovascular diseases and diabetes mellitus were most often present in the anamnesis of the patients (90).

The occurrence of a hyponatremic episode is greatly facilitated by the combined presence of triggering factors, such as excessive fluid consumption in the summer in addition to usual medication or the combined effect of medications that cause hyponatremia. Based on literature data, using other drugs predisposing to hyponatremia also increases the risk of the disease (ARBs, ACE inhibitors, non-thiazide diuretics, NSAIDs, antidepressants) (90).

Examining the change in the water spaces, the TBW increased after treatment, likely attributed to diuretic withdrawal. During treatment, ICW increased as well, but ECW did not change significantly. Different results were obtained by Filippone et al., who examined 150 patients admitted with thiazide-induced hyponatremia. The majority of patients were clinically euvolemic with increased ECW volume (91).

The relevant ion levels and, consequently, the osmolarity - per the literature data - increased significantly due to the treatment (92).

It was striking that hypokalemia also occurred frequently in addition to hyponatremia. The potassium-losing effect of thiazide and thiazide-type diuretics is exerted by inhibiting sodium reabsorption in the kidney's proximal and distal convoluted channels, so increased sodium reabsorption in the collecting duct promotes the tubular secretion of potassium (and hydrogen ions). Other researchers described similar results; in addition to severe hyponatremia, hypokalemia (average serum K^+: 3.3 mmol/L) and reduced osmolarity (average serum osmolarity: 242 mOsm/kg) were observed (93).

There was a significant reduction in eGFR, and uric acid levels increased significantly after treatment.

The reduction of stress can explain the significant decrease in cortisol after treatment. In a 2017 study, Burst et al. examined patients admitted for thiazide-associated hyponatremia, and in 125 patients, the authors also registered elevated cortisol levels (94).

No significant changes were found for the other laboratory parameters.

During our correlation tests, sodium levels were positively correlated with potassium, chloride, creatinine, uric acid, and osmolarity and negatively correlated with eGFR.

The potassium level was positively correlated with sodium, chloride, carbamide, creatinine, uric acid levels, and osmolarity and negatively with eGFR.

Examining hormone levels, renin had a positive relationship with aldosterone and a negative with urine sodium. Aldosterone had a positive correlation with renin and uric acid and a negative correlation with urine sodium. ACTH correlated positively with creatinine and negatively with eGFR. In addition, cortisol is negatively associated with sodium, potassium, chloride, and osmolarity.

SUMMARY

Based on our results so far, urine sodium did not significantly help us determine the etiology of hyponatremia. This was usually achieved without difficulty and was most often a thiazide-type diuretic, which is not sufficiently emphasized in the medical public, explaining the recurrence of potentially fatal episodes.

In milder cases, moderate fluid restriction and close follow-up are warranted, and in more severe cases, permanent avoidance of thiazide-type diuretics. Instead, furosemide and spironolactone are recommended, which cause hyponatremia less often. This is especially important for high-risk groups (older women, lower body weight, high fluid intake, low-normal sodium and potassium levels). As the disease usually manifests within 1-2 weeks, carrying out a laboratory test 2 weeks after the treatment is justified. If the sodium level approaches the bottom of the normal range, the diuretic administration should be suspended. However, it can develop in individuals who are susceptible to it, even in a few days, and the laboratory test must be performed 1-2 days after the treatment (93).

It is worth discussing fluid intake for those with hyponatremic episodes. Their attention should be drawn to the fact that, in their case, excessive intake can be harmful; they should only drink as much as it quenches their thirst (95).

The reduction of the serum cortisol level may be related to the settlement of the stress state.

The paradoxical decrease of the intracellular water space in hyponatremia is a literary novelty that requires further analysis, just like other parameters of the salt-water balance.

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SCIENTOMETRICS

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- English language papers: 7

Impact factor (up to 25-Aug-2023 based on MTMT2):

- First author: 9.100
- Cumulative: 26.090

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- Cumulative: 34

List of publications:

First author papers upon which this thesis relies:

1. Gergics M, Pham-Dobor G, Horváth-Szalai Z, Kőszegi T, Mezősi E, Bajnok L. Secondary hormonal alterations in short-term severe hypothyroidism; in the focus: Apelin and copeptin. Front Endocrinol (Lausanne). 2022 Sep 14;13:981891. doi: 10.3389/fendo.2022.981891. PMID: 36187132; PMCID: PMC9519179. Q1; IF: 6.055 (2021)
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1. Pham-Dobor G, Hanák L, Hegyi P, Márta K, Párniczky A, Gergics M, Sarlós P, Erőss B, Mezősi E. Prevalence of other autoimmune diseases in polyglandular autoimmune syndromes type II and III. J Endocrinol Invest. 2020 Sep;43(9):1-9. doi: 10.1007/s40618-020-01229-1. Epub 2020 Aug 17. PMID: 32227311; PMCID: PMC7431444.
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Secondary hormonal alterations in short-term severe hypothyroidism; in the focus: Apelin and copeptin

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Objective: This study aimed to investigate the complex interactions of thyroid hormone, apelin, and copeptin in the fluid–ion homeostasis of patients with severe transitory hypothyroidism.

Methods: In this prospective observational study, 39 patients (ECOG: 0; 11 men, 28 women, mean age: 50.3 $\pm$ 14.9 years) were investigated during short-term severe hypothyroidism due to surgical removal of the thyroid gland and after adequate thyroid replacement therapy. In addition to the routinely available lab tests, copeptin and apelin levels were determined using ELISA.

Results: In the hypothyroid state, apelin concentration was lower, while copeptin levels did not differ compared to the euthyroid condition. Apelin showed a positive correlation with copeptin (p = 0.003), sodium (p = 0.002), NT-proBNP (p < 0.001), and fT4 (p < 0.001) and a negative correlation with thyroid-stimulating hormone (TSH) (p < 0.001). In multivariate linear regression models, copeptin and TSH proved to be significant independent predictors of apelin levels, of which TSH had an explanatory power of 48.7%. Aside from apelin, copeptin only correlated with sodium (p = 0.046). Sodium levels were negatively associated with TSH (p = 0.004) and positively with ACTH (p = 0.002) and cortisol (p = 0.047), in addition to copeptin. None of the parameters were independent predictors of serum sodium levels in a multivariate regression model.

Conclusions: In short-term severe hypothyroidism, serum apelin level is markedly decreased, which may predispose susceptible patients to hyponatremia, while the level of copeptin is unchanged. TSH and copeptin are independent predictors of apelin concentration, of which TSH is stronger.

KEYWORDS
TSH, DTC (differentiated thyroid cancer), apelin, copeptin, hypothyroidism

Introduction

Hyponatremia, defined as plasma sodium concentration below 135 mmol/L, is the most common electrolyte disorder in hospitalized patients (1). Various conditions have been associated with hyponatremia, including chronic heart failure, chronic kidney disease, liver cirrhosis, hypovolemia, and the syndrome of inappropriate antidiuresis (SIAD), in which antidiuretic hormone (ADH)/arginine vasopressin (AVP) secretion occurs in the absence of an osmotic or hemodynamic abnormality (1, 2). Fluid–electrolyte imbalances, including impaired free water excretion, are common in hypothyroidism. Hyponatremia may evolve in this state; however, the exact mechanism and prevalence are not fully elucidated. ADH/AVP is released from the posterior pituitary due to increasing plasma osmolality and various stressors such as hypovolemia, hypoxia, acidosis, and severe infections (3). Hypovolemia is the main stimulus for ADH/AVP secretion, even in the presence of reduced serum osmolarity when hyponatremia may evolve. ADH/AVP acts on vasopressin-2 receptor (V2-R) in the collecting duct of the kidney to increase cAMP production and aquaporin-2 (AQP2) insertion into the apical membrane, leading to water reabsorption. Non-osmotic elevation of ADH/AVP has been related to cardiac fibrosis and dysfunction (4, 5). Some authors demonstrated an elevated level of ADH/AVP in hypothyroid patients that could be restored by thyroid hormone replacement (4, 6). In contrast, others found lower ADH/AVP in myxedematous patients (7), explaining hyponatremia with an ADH-independent mechanism of decreased water excretion. This could be the result of hypothyroidism-related kidney dysfunction with decreased glomerular filtration rate (GFR) and altered tubular activities (4, 8, 9). Methodological problems may partially explain these conflicting results since the measurement of ADH/AVP needs competitive assays due to the small size of ADH/AVP and is less reliable than the measurement of copeptin (10). Most of the ADH/AVP in the blood is bound to platelets (11), and prolonged storage of unprocessed blood samples may falsely elevate ADH/AVP levels (10, 11). Furthermore, ADH/AVP is unstable in aliquots when stored at −20°C or −80°C (12). Thus, routine measurement of circulating ADH/AVP levels could never be implemented in clinical patient care (10). Although copeptin is a more reliable humoral marker of ADH/AVP production (10), its alterations have not been investigated in hypothyroidism.

In addition to the elevation of copeptin levels, the altered secretion of further humoral factors may also play a role in the development of hyponatremia in SIAD. For example, the sex- and age-adjusted plasma levels for apelin and copeptin are 26% and 75% higher, respectively, in SIAD patients compared to healthy subjects (1, 13). The plasma apelin/copeptin ratio lies outside of the predicted range in 86% of SIAD patients. This finding emphasizes the primary osmoregulatory defect in these patients, where the hyponatremia is worsened by the inappropriately low plasma apelin concentration that cannot compensate for the increased ADH/AVP release (13).

Hypothalamic release of apelin is oppositely regulated by the plasma osmolality compared to ADH/AVP in animal and human models (14). Apelin is particularly abundant in the hypothalamic supraoptic and paraventricular nuclei, where it colocalizes with ADH/AVP in magnocellular neurons (15–18), blocking the ADH/AVP secretion. Apelin increases aqueous diuresis by increasing renal blood flow and by counteracting the antidiuretic effect of ADH/AVP on the distal convoluted and collecting tubules of the kidney (1, 19). Thus, apelin seems to play a key role in maintaining the fluid homeostasis. A lower apelin level may exert less blocking effects on the secretion and action of ADH/AVP.

In physiological conditions, ADH/AVP and apelin are released in balanced proportions from the magnocellular ADH/AVP neurons at levels appropriate for the current plasma osmolality (1). Following water deprivation, ADH/AVP is released from magnocellular vasopressinergic neurons into the bloodstream more rapidly than it is synthesized, causing the depletion of ADH/AVP in magnocellular neurons. In the meantime, the release of apelin decreases and apelin accumulates in magnocellular neurons. Thus, after dehydration, ADH/AVP and apelin are regulated in opposite manners, to facilitate systemic ADH/AVP release and suppress diuresis.

Following water loading, ADH/AVP release is decreased from magnocellular vasopressinergic neurons, causing an accumulation in ADH/AVP, while apelin release increases, leading to a depletion of apelin in magnocellular neurons. Thus, after water loading, ADH/AVP and apelin are regulated in opposite manners, to facilitate systemic apelin release and to increase aqueous diuresis.

The transcription of preproapelin, the precursor of the peptide hormone apelin, is present in other sites of the central nervous system, with the highest levels in the thalamus and frontal cortex (16, 20–22). Furthermore, apelin and/or its receptor can be found in tissues such as the placenta, heart, lung, kidney, liver, gastrointestinal tract, adrenals, uterus, ovaries (23–26), endothelial cells of various vessels, and plasma cells (27–29). Moreover, the expression of apelin increases during adipocyte differentiation (30). Apelin also plays a major role in blood pressure regulation as an endothelium-dependent

Abbreviations: ACTH, adrenocorticotropic hormone; ADH, antidiuretic hormone; ANH, atrial natriuretic hormone; AVP, arginine vasopressin; BIA, bioelectrical impedance analysis; BMI, body mass index; DTC, differentiated thyroid cancer; eGFR, estimated glomerular filtration rate; fT3, free triiodothyronine; fT4, free thyroxin; GFR, glomerular filtration rate; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; RAI, radioiodine; RAS, renin–angiotensin system; SIAD, syndrome of inappropriate antidiuretic hormone secretion; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

vasodilator and has a positive inotropic effect by increasing cardiac output (19, 31). Hypoxia leads to the release of hypoxia-inducible factor (HIF-1) that upregulates the apelinergic signaling. However, it is not known how hypovolemia itself modulates the apelinergic system (1, 31).

Another humoral alteration demonstrated in hypothyroidism, paradoxically to the extracellular volume expansion, is the reduced level of atrial natriuretic hormone (ANH) (4, 32). Furthermore, patients with hypothyroidism often have lower plasma renin activity and plasma aldosterone concentrations (4) which can be explained as a humoral response to extracellular fluid retention.

Our study aimed to investigate the rather complex interactions of these humoral factors in the fluid–ion homeostasis in patients with severe transitory hypothyroidism.

Materials and methods

Patients

Patients between the age of 18 and 75 years with differentiated thyroid cancer (DTC) were included. Patients had no metastases and were scheduled for radioiodine (RAI) therapy after total or near-total thyroidectomy in this prospective, observational study. The patients were consecutively enrolled from 12 January 2018 to 21 February 2020.

They were in a complete endogenous or exogenous levothyroxine deficiency for at least 4 weeks before sampling. Hypothyroidism was confirmed in all patients by the determination of low serum free thyroxine (fT4), low serum free triiodothyronine (fT3), and markedly elevated thyroid-stimulating hormone (TSH). Patients were prospectively evaluated on the day of RAI therapy and on thyroxine 10–12 weeks later. DTC was not advanced (ECOG: 0). The exclusion criteria were any known chronic diseases or medications that could potentially interfere with thyroid status, including abnormal fluid retention due to heart, liver, or kidney diseases or diuretic treatment. Between the hypothyroid and control investigations, there were no i) changes in the medications beyond the thyroxine supplementation and ii) significant intercurrent diseases. During the 10–12 weeks follow-up, patients took their regular medications before control laboratory tests in the morning, including levothyroxine supplementation.

Study protocol/design

Venous blood samples were obtained between 8 and 10 a.m. for further biochemical analysis. After that, body weight was measured to the nearest kilogram and height to the nearest centimeter. The waist circumference was gauged when the participant was standing upright with a tape measure in the horizontal plane midway between the lowest rib and the iliac crest, while the hip circumference was measured over the most pronounced area of the buttocks. The same investigator made all the anthropometric measurements to avoid interindividual variations.

Bioelectrical impedance analysis (BIA) (Bodystat Quadscan 4000, Bodystat Ltd., P.O. Box 50. IM99 1DQ Douglas, Isle of Man, United Kingdom) was used for the assessment of body composition. Patients were placed in the supine position for at least 5 min before the measurement. All participants had light clothing and their earrings, rings, bracelets, and any other metal were removed, which could influence the measurement results. Every measurement took approximately 30 s for each participant.

Only patients fulfilling the following BIA protocol were included: no alcohol consumption for 48 h, no strenuous activity for 12 h, and fasting for at least 4 h before the test. Patients with implanted electronic devices, like heart pacemakers, were excluded.

The study was carried out by the Declaration of Helsinki (2000) of the World Medical Association and approved by the Ethics Committee at the Medical Center of the University of Pécs (6961/2017). Subjects participated in the study after their written informed consent was obtained.

Determination of routine laboratory tests and neurohormonal mediators

Venous blood samples were taken into plain tubes with accelerator gel and EDTA tubes (Vacutainer®, Becton Dickinson, Eysins, Vaud, Switzerland). Sera and plasma samples were obtained by centrifugation for 20 min at 2,300 rpm, then aliquoted in Eppendorf tubes, and stored at −80°C until analysis.

Chemistry panel, endocrine parameters, and fully automated blood picture tests were determined using the standard laboratory diagnostic kits and automated instrumentation of the Department of Laboratory Medicine, University of Pécs (accreditation number: NAH-1-1553/2016). The reference range for TSH was 0.27–4.2 mU/L, and for fT4, it was 12.0–22.0 pmol/L. Serum apelin and copeptin levels were measured with the ELISA method using Human Apelin ELISA kit (Catalog No. abx585113, Abbexa Ltd., UK, intra-assay CV <10%, inter-assay CV <10%), and Human Copeptin (CT-proAVP) ELISA kit (Catalog No. abx252269, Abbexa Ltd., UK, intra-assay CV <10%, inter-assay CV <10%) according to the manufacturers' instructions on a BioTek Synergy HT plate reader at 450 nm. Serum N-terminal prohormone of brain natriuretic peptide (NT-proBNP) levels were measured using an immunoassay method using the Immulite 2000 automated instrument (Catalog No. L2KNT2, LNTCM, Siemens Healthcare Diagnostics Inc., USA).

Statistical analysis

The Shapiro–Wilk test was used to check normal distributions on continuous variables. Data are presented as mean ± SD in parameters with normal distribution, while median and interquartile values are in the non-normal distribution. Comparisons of the two phases were made with paired sample *t*-test and Wilcoxon signed-rank test in normal and non-normal distributions, respectively. In the pairwise comparison, we applied the Bonferroni correction of the *p*-values to decrease the type I error. Correlation analysis calculating the Spearman coefficient was performed to assess the strength of the association between the different variables. Multiple regressions were used to determine the associations between clinical variables and serum concentrations of copeptin and apelin. The factors that were significantly associated with the single outcome at the univariate analysis and those considered to be biologically conceivable were included in multiple regressions. Statistical significance was assumed at p <0.05. All analyses were performed with SPSS (version 22.0, SPSS Inc., Chicago, IL, USA).

Results

A total of 39 patients (11 men and 28 women) with a mean age of 50.28 ± 14.90 years were evaluated. Anthropometric and biochemical variables before and after correction of hypothyroidism are shown in Table 1. None of the patients had significant peripheral edema retention, ascites, or pleural effusion. The mean of the applied thyroxine dose for the correction of hypothyroidism was 139.74 ± 31.27 µg. The apelin level was 38% lower in the hypothyroid state than post-treatment, while copeptin levels did not change. Furthermore, serum sodium, chloride, potassium, estimated glomerular filtration rate (eGFR), NT-proBNP, adrenocorticotropic hormone (ACTH), and renin activity were significantly lower in the hypothyroid state. At the same time, BMI, abdominal circumference, and total body fat mass were higher during hypothyroidism. There was no difference in the extracellular fluid, intracellular fluid, serum osmolality, or urinary osmolality values. In the hypothyroid phase, hyponatremia was only detected in two patients (5.1%) in mild form and none of the euthyroid patients.

The relations of apelin and copeptin to each other and selected other variables are shown in Figures 1–3 and Tables 2–5. During the assessment of correlations, to broaden the investigated biochemical parameters, the whole database was used. Apelin and copeptin correlated with each other and serum sodium concentrations. Copeptin was not associated with any other investigated parameter, while apelin positively correlated with sodium, chloride, potassium, NT-proBNP, and fT4 and negatively correlated with TSH. Neither apelin nor copeptin was related to anthropometric variables, including BMI, fat mass, or

TABLE 1 Comparisons of selected anthropometric and biochemical characteristics between the two study phases.

Parameters	Hypothyroid phase	Control phase	*p*-value
Waist circumference (cm)	**94.13 ± 14.70**	**93.46 ± 14.50**	**0.039**
Body mass index	**29.10 (25.10–32.10)**	**28.20 (24.70–32.30)**	**<0.001**
Fat mass (kg)	**27.50 (21.40–32.30)**	**24.20 (18.00–31.90)**	**0.001**
Total body fluid (L)	38.30 (34.10–46.20)	37.80 (34.20–45.90)	0.624
Extracellular fluid (L)	16.80 (15.10–19.70)	17.30 (15.50–20.20)	0.673
Intracellular fluid (L)	22.30 (19.40–28.20)	22.40 (18.90–29.50)	0.451
TSH (mU/L)	**75.78 ± 25.28**	**2.45 ± 4.12**	**<0.001**
fT4 (pmol/L)	**2.93 ± 1.70**	**24.90 ± 5.71**	**<0.001**
Na (mmol/L)	**140.77 ± 2.60**	**142.33 ± 1.75**	**0.002**
Cl (mmol/L)	**100.82 ± 3.44**	**102.50 ± 2.63**	**0.012**
K (mmol/L)	**4.28 ± 0.35**	**4.41 ± 0.34**	**0.025**
eGFR (ml/min/1.73 m^2)	**77.00 (61.00–90.00)**	**90.00 (72.00–90.00)**	**<0.001**
Uric acid (µmol/L)	295.28 ± 84.92	287.15 ± 91.77	0.363
Serum osmolality (mOsmol/kg)	287.49 ± 14.66	286.85 ± 7.14	0.817
Urine osmolality (mOsmol/kg)	530.79 ± 257.03	602.67 ± 253.90	0.136
Apelin	**1,901.88 ± 766.67 pg/ml = 1,226.39 ± 494.37 fmol/ml**	**3,080.65 ± 516.99 pg/ml = 1,986.49 ± 333.37 fmol/ml**	**<0.001**
Copeptin	1,007.7 (626.0–1,822.8) pg/ml = 243.7 (151.4–440.8) pmol/L	933.9 (619.6–1,437.4) g/ml = 225.8 (149.8–347.6) pmol/L	0.615
NT-proBNP (pg/ml)	**36.78 (20.59–73.73)**	**69.58 (37.37–194.92)**	**<0.001**
Renin activity (ng/ml/h)	**0.70 (0.42–1.63)**	**1.57 (0.92–2.85)**	**0.003**
Aldosterone (pg/ml)	166.50 (112.22–221.27)	174.23 (156.90–268.80)	0.18
ACTH (pg/ml)	**11.9 (10.00–16.00)**	**16.40 (12.20–22.50)**	**0.001**
Cortisol (nmol/L)	318.80 (242.30–385.90)	348.00 (275.60–440.20)	0.283

Significant differences are labeled as bold.

body fluid values, and they did not differ among genders. In 10 patients, fT4 levels increased above the reference range due to the routinely taken thyroxine supplementation. None of the investigated parameters were different in this subpopulation compared to the rest of the patients (beyond TSH and fT4 levels). Regarding relevant humoral factors for fluid–ion homeostasis, changes in hypothyroidism and their correlations are summarized in Table 3.

Linear regression tests were run to test the potential independent predictors of either serum apelin or copeptin concentrations. The most representative results are shown in Tables 4 and 5. To widen the range of tested parameters during the regression analyses, they were carried out in the pooled data. Copeptin and TSH levels were significant determinants of serum apelin levels. TSH was especially strong, explaining almost half of the variance of the apelin concentrations. Regarding copeptin levels, aside from apelin, TSH was also an independent predictor, even though in a univariate test, no correlation was detected between them. However, TSH was a weaker determinant of copeptin concentrations than apelin (12.3% compared to 48.7%).

Serum sodium level had a variance of 5.47 mmol/L and, in addition to apelin and copeptin, was associated with the concentrations of TSH (r = −0.321; p = 0.004), ACTH (r = 0.349; p = 0.002), and cortisol (r = 0.225; p = 0.047). However, none of them were independent determinants of serum sodium levels in multiple regression analyses (data not shown).

Discussion

Conflicting results were reported in the literature about apelin levels in hypothyroid patients. While some authors did not find a significant difference in subclinical or manifested hypothyroid patients (28, 33), others demonstrated lower apelin levels in subclinical hypothyroidism restored by levothyroxine supplementation (34). We can confirm these latter observations as we found significantly decreased apelin levels in our hypothyroid population. To the best of our knowledge, our investigation is the first one analyzing the relations of apelin to other humoral regulators of fluid–electrolyte homeostasis and the components of thyroid dysfunction. Of the investigated parameters, copeptin and TSH levels turned out to be predictors of serum apelin levels, with TSH being a much stronger determinant. In contrast, thyroxine levels did not have an impact.

In our study, hyponatremia was uncommon in hypothyroidism affecting only 5% of the patients. However, serum sodium concentrations were significantly lower in the hypothyroid phase, correlated with both apelin and copeptin levels in univariate analyses, but neither apelin nor copeptin levels were independent predictors of serum sodium, and vice versa, serum sodium was not an independent predictor of either apelin or copeptin levels. This might be explained by the relatively low variance of the serum sodium levels (i.e., 5.47 mmol/L) of our patients with free fluid intake compared to earlier studies investigating the effect of hypertonic saline infusion or water loading test (14). Interestingly, the alterations mentioned above were not present in the measured body fluid, serum, and urinary osmolality values.

Hyponatremia was rare and no alteration was detected in the ADH/AVP secretion (reflected by copeptin levels) of our patient population with short-term severe hypothyroidism. However, it can be hypothesized that a hypothyroidism-related decrease in apelin levels may contribute to the development of hyponatremia in some susceptible hypothyroid patients;

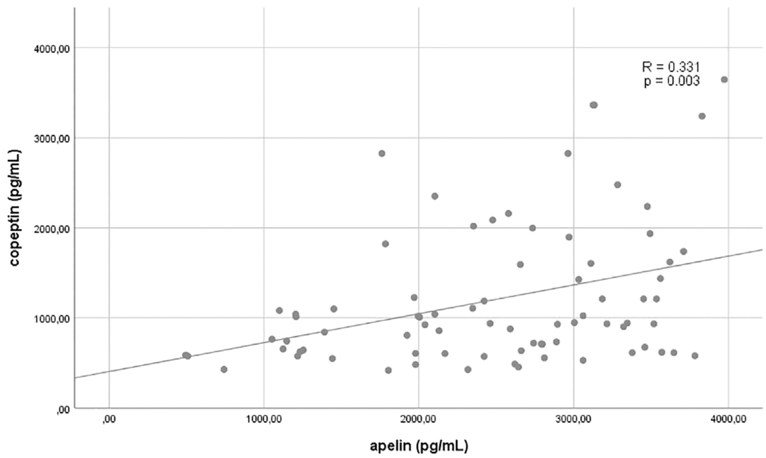

FIGURE 1
Correlation between copeptin and apelin.

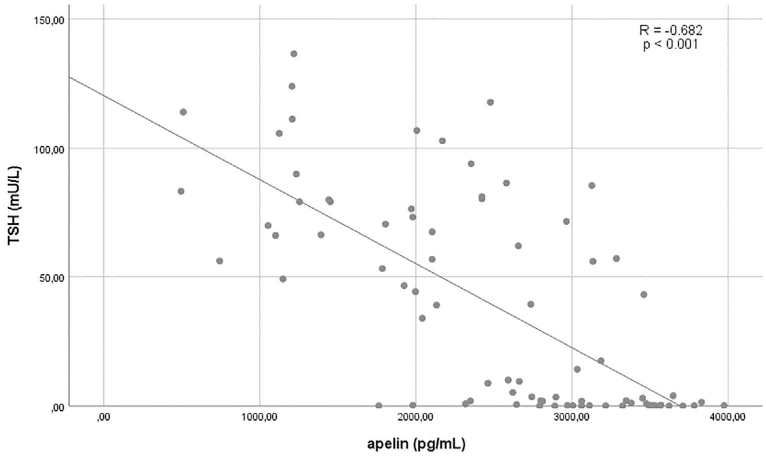

FIGURE 2
Correlation between TSH and apelin.

namely, if simultaneous ADH/AVP oversecretion also evolves, for example as a side effect of a medication, due to high external temperature or increased fluid intake, hyponatremia may become manifested. The lower apelin levels may also contribute to the other comorbidities of hypothyroidism, e.g., accelerated atherosclerosis (31, 34).

There are conflicting data in the literature about ADH/AVP level changes in hypothyroid patients (4–7). This may be the consequence of the heterogeneity of the patient populations. Some authors only investigated young women with long-term hypothyroidism without any comorbidities (4). In chronic hypothyroidism, non-osmotic stimulation of ADH/AVP secretion by cardiac fibrosis and dysfunction may explain elevated serum ADH/AVP levels (4, 5). These alterations may be absent in short-term forms of hypothyroidism like in our model. In addition to the different patient populations, discordant results may be due to technical difficulties in measuring ADH/AVP levels. At the same time, copeptin is a more reliable surrogate marker of ADH/AVP (10). To the best of our knowledge, our study was the first to investigate the copeptin levels in hypothyroidism.

In our patients, copeptin and apelin had positive correlations with each other and the serum sodium level, contradictory to the expected reciprocal changes in copeptin and apelin plasma concentrations. Moreover, copeptin and apelin levels were significant independent determinants of each other's serum concentrations during multiple regression analyses. Furthermore, TSH was also a predictor of both copeptin and

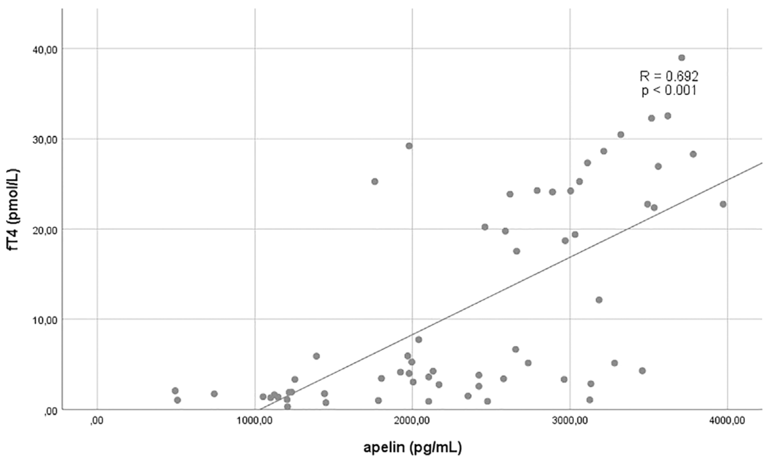

FIGURE 3
Correlation between fT4 and apelin.

TABLE 2 Relations of apelin and copeptin to the selected anthropometric and biochemical parameters.

Parameters	*R* values for apelin	*p*-value	*R* values for copeptin	*p*-value
Age	0.077	0.504	0.075	0.512
Waist circumference	0.004	0.969	0.035	0.761
Body mass index	−0.039	0.737	0.043	0.706
Fat mass	0.064	0.576	0.071	0.535
Total body fluid (L)	−0.198	0.082	0.096	0.403
Extracellular fluid (L)	−0.103	0.369	0.199	0.080
Intracellular fluid (L)	−0.173	0.130	0.063	0.586
TSH	**−0.682**	**<0.001**	−0.066	0.568
fT4	**0.692**	**<0.001**	0.067	0.599
Na	**0.349**	**0.002**	**0.226**	**0.046**
Cl	**0.451**	**<0.001**	0.186	0.115
K	**0.271**	**0.017**	0.176	0.122
eGFR	0.175	0.125	−0.131	0.251
Uric acid	−0.173	0.130	0.142	0.216
Serum osmolality (mOsmol/kg)	−0.031	0.789	0.155	0.177
Urine osmolality (mOsmol/kg)	0.091	0.426	−0.008	0.945
Apelin	1	1	**0.331**	**0.003**
NT-proBNP	**0.390**	**<0.001**	0.012	0.914
Renin activity	0.088	0.446	0.006	0.956
Aldosterone	0.097	0.402	0.113	0.326
ACTH	0.177	0.120	0.112	0.328
Cortisol	0.037	0.744	0.045	0.692

Significant associations are labeled as bold.

apelin levels of which the association with apelin was especially strong, explaining almost half of its variance. Meanwhile, T4 was not a significant variable in these models. These findings may suggest that in this clinical setting the potential alterations of these hypothalamic hormones are not basically related to abnormalities in the osmotic or volemic state, but instead are regulated by hypothyroidism. Disturbances in the hypothalamic regulation may explain these findings, e.g., increased TRH secretion being present in hypothyroidism might be responsible for the low apelin level in this state.

Apelin or copeptin levels did not show associations with adrenal cortical hormones, and only the former was correlated to NT-proBNP concentrations. In line with previous observations of reduced ANH levels in the hypothyroid state (4, 32), we found significantly lower NT-proBNP concentrations. However, aldosterone concentrations were not reduced in our population, opposite to earlier findings in hypothyroid patients or healthy men challenged by hypertonic solution (4, 14). At the same time, renin activities were significantly elevated during the normalization of hypothyroidism, just like in previous studies (4, 35). The 89% elevation of NT-proBNP levels measured following the correction of hypothyroidism may at least partially explain the lack of significant changes in aldosterone concentrations. The aldosterone secretion stimulating effects of the increased renin activity and potassium concentrations may be counterbalanced by the simultaneous elevation of ANH levels

TABLE 3 Summary of relevant humoral factors for fluid–ion homeostasis; changes in hypothyroidism compared to the corresponding levels after thyroid hormone replacement and their correlations.

Humoral factor	Level in hypothyroidism	*R* values of correlations			
		Apelin	Copeptin	NT-proBNP	Aldosterone
Apelin	**−38%**	1	**0.331****	**0.390*****	0.097
Copeptin	+7%	**0.331****	1	0.012	0.113
NT-proBNP	**−48%**	**0.390*****	0.012	1	−0.087
Aldosterone	−5%	0.097	0.113	−0.087	1
Cortisol	−9%	0.037	0.045	−0.023	**0.331****

Significant values are labeled as bold (**: p < 0.01, ***: p < 0.001).

TABLE 4 A linear regression model with apelin as a dependent variable.

	Predictive power (%)	*p*-value
(Constant)		0.575
Age	0	0.869
Sex	1.5	0.114
BMI	1.4	0.441
Na	0.8	0.306
Copeptin	**13**	**<0.001**
TSH	**48.7**	**0.007**
fT4	1.3	0.184
NT-proBNP	0	0.890

Significant associations are labeled as bold.

TABLE 5 A linear regression model with copeptin as a dependent variable.

	Predictive power (%)	*p*-value
(Constant)		0.582
Age	0.5	0.530
Sex	0.1	0.835
BMI	2	0.146
Na	0	0.784
TSH	**12.3**	**0.001**
Apelin	**13.1**	**<0.001**

Significant associations are labeled as bold.

(measured as NT-proBNP) during the correction of hypothyroidism. Moreover, the increase in apelin levels following the correction of hypothyroidism may contribute to the elevation of NT-proBNP due to its hemodynamic effects.

Understanding the regulation of apelin secretion is key as it plays a pivotal role in obesity, diabetes, cancer, heart failure, increasing cardiac output, and hypoxia-related diseases attenuating oxidative stress. Moreover, this peptide can be treated as a biomarker for cardiovascular diseases and a protector against apoptosis (31).

The greatest advantage of our study is that multiple potentially interrelated humoral factors were evaluated in a homogeneous patient population without comorbidities that could distort the obtained results. We attempted to understand the potential pathomechanisms of our basic observations by applying various statistical methods. However, this approach has inherent limitations, and therefore, multiple findings cannot be reliably explained. Another shortcoming of our study is the lack of a normal control population. However, matching controls in multiple parameters beyond usual anthropometric ones would have been difficult, and the self-control pattern of our study allowed us to achieve more or less standardized experimental conditions. Furthermore, our population consisted of relatively few participants. However, many of our original observations and relationships were statistically highly significant.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee at the Medical Center of the University of Pécs (6961/2017). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MG, EM, and LB designed the study, analyzed the data, and wrote the manuscript. MG measured the body composition and conducted the statistical analysis. GP-D participated in the evaluation of laboratory results. ZH-S carried out the ELISA tests. TK supervised the laboratory investigations. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Article

Apelin-13 as a Potential Biomarker in Critical Illness

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Abstract: Background: The adrenocortical system and copeptin as prognostic markers were intensively investigated in critical illness. The potential predictive power of apelin-13 as a biomarker is largely unknown. We aimed to investigate the prognostic role of apelin-13 in relation to free cortisol, aldosterone, CRH, and copeptin in critically ill patients. Methods: In this prospective observational study, 124 critically ill patients (64 men, 60 women, median age: 70 (59–78) years) were consecutively enrolled at the time of admission. All routinely available clinical and laboratory parameters were evaluated and correlated to hormonal changes. Results: Serum apelin-13 was 1161 (617–2967) pg/mL in non-survivors vs. 2477 (800–3531) pg/mL in survivors ($p = 0.054$). The concentrations of apelin-13 and CRH had strong positive correlations ($r = 0.685$, $p < 0.001$) and were significantly higher in surviving non-septic patients (Apelin-13 (pg/mL): 2286 (790–3330) vs. 818 (574–2732) $p < 0.05$; CRH (pg/mL) 201 (84–317) vs. 89 (74–233) $p < 0.05$). Apelin-13 and free cortisol were independent determinants of survival in the multivariate Cox regression analysis, while copeptin, CRH, or aldosterone were not. Conclusions: Beyond free cortisol, serum apelin-13 may also help refine prognostic predictions in the early phase of critical illness, especially in non-septic patients.

Keywords: copeptin; apelin-13; free cortisol; CRH; critical illness

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1. Introduction

1.1. Background

The mortality of patients requiring critical care is high and partly unpredictable. Complex prognostic scores have been created to improve the prediction of outcomes. However, despite intensive efforts, the predictive power of prognostic biomarkers is not optimal. Looking for new biomarkers would be essential not just to improve the prognostic role but for a better understanding of the pathomechanisms of critical conditions as well [1,2].

The prognostic roles of cortisol and arginine vasopressin (AVP) have been intensively studied in the literature.

Neuro-hormonal responses, including elevating cortisol levels, play a vital role in stress. Free cortisol concentration is typically high in critically ill patients due to the early activation of the hypothalamus-pituitary–adrenocortical axis (HPA) and decreased cortisol metabolism [3,4]. Previously, we demonstrated that free cortisol is an independent predictor of 30-day mortality in a mixed population of critically ill patients admitted to the ICU [5]. In addition to its prognostic value, free cortisol was reported to be a good marker of inflammatory response in septic shock [6].

Arginine vasopressin, or anti-diuretic hormone (ADH), is released from the posterior pituitary by increasing plasma osmolality and upon various stressors, such as hypovolemia, hypoxia, acidosis, and severe infections. The copeptin level is a reliable surrogate parameter for the AVP effect in healthy and critically ill patients [7]. Copeptin is elevated in patients admitted to the ICU, e.g., in a septic state [7–9]. Moreover, copeptin also serves as a prognostic marker; at admission, copeptin serum concentration is higher in non-survivors [10,11], and it is closely associated with severity scores (SAPS II and APACHE II) [12]. Copeptin is an independent predictor of survival in septic shock [7,13] and acute heart failure [14]. The apelinergic system is closely related to AVP and other stress reactions, but its potential role as a prognostic biomarker is largely unknown.

Apelin, a 36-aminoacid peptide hormone, is particularly abundant in the supraoptic and paraventricular nuclei, co-localizing with AVP in magnocellular neurons [15,16]. Apelin blocks the AVP secretion and action on the distal convoluted and collecting tubules of the kidney [17]. Its receptor has been detected in the cerebral cortex, hypothalamus, hippocampus, and pituitary gland [15]. Furthermore, the apelinergic system can be found in peripheral tissues: in the heart [18], gastrointestinal tract, skeletal muscle, liver, ovary, kidney, adipose tissue, lung, and endothelial cells [19].

In animal models, apelin's in vivo effects on HPA function have been mediated by CRH and AVP-dependent mechanisms [20]. Apelin also regulates cardiovascular homeostasis, which is essential in regulating blood pressure, increasing cardiac output, and having a cardioprotective effect against oxidative stress [18,19,21]. Moreover, apelin is an endothelium-dependent vasodilator via the renin-angiotensin system (RAS) [17,22]. In addition to its essential role in cardiovascular homeostasis, apelin is involved in fluid homeostasis, glucose metabolism, and other physiological activities [17,21].

Apelin-13 has been identified as a significant apelin isoform in human plasma [23].

Corticotropin-releasing hormone (CRH) is a central regulator of the hormonal stress response, stimulating both corticotropin (ACTH) and AVP secretion [24]. The parvocellular CRH neurons also co-express AVP, which acts as a second 'releasing factor' for ACTH and CRH [25]. In contrast to ACTH, CRH is rarely measured in peripheral blood samples because it does not correlate well with those in the hypothalamic-hypophysial portal plasma. CRH is expressed in the brain, immune cells, and the gut, where gene expression is upregulated by lipopolysaccharide (LPS) [26]. To the best of our knowledge, there is no information about the serum CRH level at admission in critically ill patients.

1.2. Objectives

Our present study aimed to investigate a more complex interplay of the hypothalamic and adrenocortical systems in a mixed population of patients with critical illness. Based on the abovementioned data, the following hormones were selected for analysis as biomarkers: apelin-13, copeptin, CRH, free cortisol, and aldosterone.

2. Materials and Methods

2.1. Study Design, Setting

A prospective cohort study was carried out on critically ill patients admitted to the Intensive Care Unit of either Department of Emergency Medicine or 1st Department of Medicine, Clinical Center, the University of Pécs. Recruitment was achieved in two periods, between June and October 2012 and May 2019 and June 2020. Vital signs, clinical status, and routine laboratory parameters were monitored. The treatment of patients was thoroughly evaluated, and blood samples disturbed by glucocorticoid treatment were excluded from further analysis. The severity of the diseases was scored according to the SAPS II and the APACHE II scoring systems.

2.2. Participants

It was a mixed population of patients with medical emergencies. The patients were consecutively enrolled in two periods requiring intensive care due to vital organ dysfunction [27].

Patients with COVID-19, surgical procedure, or trauma were excluded. None of the patients received etomidate, ketoconazole, or any other drug influencing steroid metabolism.

Eighteen patients needed complete cardiopulmonary resuscitation, and eight were defibrillated before admission.

Our study was performed by the ethical guidelines of the 2003 Declaration of Helsinki, and we obtained the permission of the Regional Research Ethical Committee of the University of Pécs. Written informed consent was obtained from the participants or the participants' parent/legal guardian/next of kin to participate in the study.

2.3. Determination of Routine Laboratory Tests and Neurohormonal Mediators

At the time of admission, the chemistry panel and fully automated blood picture tests were determined using the standard laboratory diagnostic kits and automated instrumentation of the Department of Laboratory Medicine, University of Pécs (accreditation number: NAH-1-1553/2016).

At admission, blood samples were taken to measure the free cortisol, apelin-13, copeptin, CRH, and aldosterone levels. They were collected in plastic tubes in an anticoagulant-free Vacutainer (Becton Dickinson, Hungary Kft., Környe, Hungary). After centrifuging the collected blood samples at 2200× g for 10 min, serum was separated into aliquots in Eppendorf tubes and frozen under −80 °C. Sample preparation and the measurements for free cortisol analysis were performed according to the validated method using high-performance liquid chromatography coupled with high-resolution ESI-TOF mass spectrometry described by Montsko et al. [28].

Serum apelin-13, copeptin, and CRH levels were measured with the ELISA method using Human Apelin-13 ELISA kit (Catalog No.: abx252028, Abbexa Ltd., Cambridge, UK; intra-assay: CV < 10%, inter-assay: CV < 10%), Human Copeptin (CT-proAVP) ELISA kit (Catalog No.: abx252269, Abbexa Ltd., Cambridge, UK; intra-assay: CV < 10%, inter-assay: CV < 10%), and Human Corticotropin-Releasing Hormone (CRH) ELISA Kit (Catalog No.: MBS264947, MyBioSource; intra-assay: CV $\leq$ 8%, inter-assay: CV $\leq$ 12%) according to the manufacturer's instructions on a BioTek Synergy HT plate reader at 450 nm. Serum aldosterone was measured using the radioimmunoassay method (Ref: IM1664, RIA-mat 280, Stratec).

2.4. Statistical Methods

Statistical analyses were carried out using SPSS 22.0 software. The Shapiro-Wilk test was used to check normal distribution; data are presented as mean ± SD in parameters with normal distribution, while median and interquartile values are in the non-normal distribution. To determine the relationship between parameters, Spearman's correlation was used. Comparisons of two subgroups were made with Mann-Whitney U tests. The backward selection was used for binary logistic regression analyses, and a p-value of <0.1 was deemed significant. A p-value of <0.05 was regarded as significant, except for multiple logistic regression analyses; if linear methods with backward selection were used, a p-value of <0.1 was determined as significant. The beta value reflects the direction and the power of association. Cox regression analysis with backward selection was used to describe survival time; a p-value of <0.1 was significant.

3. Results

A total of 124 patients were recruited for the study. Patients represented a mixed population with a demand for critical care.

The key parameters of the patient population are shown in Table 1.

SAPS II score and hormonal parameters according to 30-day survival are shown in Table 2. Compared to the normal range, the free cortisol, CRH, and copeptin levels were highly elevated in both surviving and non-surviving patients with critical illnesses (no information regarding the normal range of apelin-13 is available.) The median sodium, potassium, creatinine, and urea values were within the normal limit.

Table 1. Patient's main characteristics.

Age (years, median, interquartile)	70 (59–78)
Gender (male/female)	64/60
30-day mortality rate	43 of 124 35%
Mechanical ventilation	46%
Catecholamine treatment	58%
Acute hemodialysis	27%
APACHE II score (median, interquartiles)	22 (17–29)
SAPS II score (median, interquartiles)	40 (32–60)
Diagnosis	
Sepsis	30
Acute heart failure	21
Pulmonary embolism	8
Acute myocardial infarction	8
Primary respiratory failure	20
Critical arrhythmias	10
Others	27

Table 2. SAPS II score and hormonal parameters as median (Q1-Q3), according to 30-day survival in the total population.

	Total Population	Normal Range	Survived on Day 30 (n = 81; 65%)	Deceased within 30 Days (n = 43; 35%)
SAPS II score ***	40 (32–60)	not applicable	**36 (24–46)**	**60 (42–70)**
Free cortisol (nmol/L) **	35 (10–126)	1–8	**25 (5–89)**	**65 (29–199)**
Copeptin (pg/mL) *	697 (459–1106)	4–52	**642 (416–1175)**	**765 (568–1055)**
Apelin-13 (pg/mL)	2024 (704–3320)	no data	2477 (800–3531)	1161 (617–2967)
CRH (pg/mL)	176 (80–356)	4–11	205 (85–357)	105 (75–322)
Aldosterone (pg/mL)	162 (79–354)	67–335	156 (76–296)	212 (93–419)

* p-value is between 0.05 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001, bold shows significant parameters.

The median and interquartile of the investigated hormonal parameters are separately demonstrated according to the main diagnosis of admission in Table 3.

Correlations of hormonal parameters with hormonal levels, 30-day mortality, severity score, and clinical parameters are demonstrated in Table 4.

Fifty-five patients (44%) had hypotension at admission. Significant correlations were found between hypotension and free cortisol (0.328, $p < 0.001$), copeptin (0.226, $p = 0.012$), aldosterone (0.221, 0 = 0.014), CRH (0.274, $p = 0.002$), and SAPS II (0.291, $p = 0.001$), but not with apelin-13 or 30-day mortality.

While investigating determinants of serum apelin-13 level by multiple linear regression analysis in two different models, CRH, SAPS II, serum sodium, potassium, and the presence of kidney injury were independent predictors (Tables 5 and 6). Apelin-13 level was significantly elevated in patients with kidney failure (without vs. with kidney injury: 1439 (648–3250) vs. 2967 (1757–3836), $p = 0.005$). Using the same model, the independent predictors of serum CRH level were apelin-13 level and kidney injury (Table 7).

Table 3. The concentrations of hormonal parameters as median (Q1–Q3) differentiated by the underlying reasons for admission.

	Free Cortisol (nmol/L)	Apelin-13 (pg/mL)	CRH (pg/mL)	Aldosterone (pg/mL)	Copeptin (pg/mL)
Sepsis (n = 30)	117 (26–222)	2869 (1155–3766)	376 (107–801)	127 (69–395)	1566 (693–3379)
Acute heart failure (n = 21)	32 (5–150)	3107 (1254–5180)	235 (85–287)	197 (113–413)	880 (487–1479)
Pulmonary embolism (n = 8)	4 (3–8)	3037 (2503–3505)	336 (209–819)	117 (61–231)	405 (297–443)
Acute myocardial infarction (n = 8)	64 (4–117)	3434 (2825–5816)	318 (184–806)	173 (105–802)	664 (273–1632)
Primary respiratory failure (n = 20)	31 (12–72)	648 (489–2517)	89 (58–231)	227 (107–350)	652 (509–733)
Critical arrhythmias (n = 10)	26 (4–93)	2269 (823–3677)	158 (121–299)	114 (65–204)	831 (440–1385)
Others (n = 27)	36 (21–67)	732 (512–1742)	83 (70–204)	212 (67–386)	513 (449–745)

Table 4. Correlations of hormonal and severity parameters.

	Free Cortisol	Copeptin	Apelin-13	Aldosterone	CRH	SAPS II	30-Day Mortality
Free cortisol		**0.217** *	−0.105	**0.359** ***	0.098	**0.480** ***	**0.280** **
Copeptin	**0.217** *		**0.214** *	0.060	**0.251** **	0.106	**0.178** *
Apelin-13	−0.105	**0.214** *		0.006	**0.685** ***	**−0.231** **	−0.173
Aldosterone	**0.359** ***	0.060	0.006		0.028	**0.197** *	0.101
CRH	0.098	**0.251** **	**0.685** ***	0.028		−0.079	−0.124
SAPS II	**0.480** ***	0.106	**−0.231** **	**0.197** *	−0.079		**0.510** ***
30-day mortality	**0.280** **	**0.178** *	−0.173	0.101	−0.124	**0.510** ***	

* *p*-value is between 0.05 and 0.01, ** *p*-value is between 0.01 and 0.001, *** *p*-value is below 0.001, bold shows significant parameters.

Table 5. Determinants of serum apelin-13 level by multiple linear regression analysis.

Dependent variable: Apelin-13	
Investigated Parameters	**Beta-Value**
CRH ***	**0.405**
SAPS II *	**−0.197**
Sodium *	**−0.152**
Potassium *	**−0.196**
Age *	**0.160**
Free cortisol	−0.025
Copeptin	0.122
Aldosterone	−0.050
Creatinine	0.092
Urea	0.190
Sex	0.016
Sepsis	−0.122
R-squared	0.334
Adjusted R-squared	0.262

* *p*-value is between 0.1 and 0.01, *** *p*-value is below 0.001, bold shows significant parameters.

Table 6. Determinants of serum apelin-13 level by multiple linear regression analysis.

Dependent variable: Apelin-13	
Investigated Parameters	**Beta-Value**
CRH ***	**0.330**
SAPS II *	**−0.281**
Sodium *	**−0.142**
Age *	**0.211**
Kidney injury *	**0.263**
Potassium	−0.157
Free cortisol	−0.060
Copeptin	0.064
Aldosterone	−0.021
Creatinine	0.048
Urea	0.034
Sex	0.028
R-squared	0.361
Adjusted R-squared	0.292

* p-value is between 0.1 and 0.01, *** p-value is below 0.001, bold shows significant parameters.

Table 7. Determinants of serum CRH level by multiple linear regression analysis.

Dependent Variable: CRH	
Investigated Parameters	**Beta-Value**
Apelin-13 ***	**0.374**
Kidney injury *	**0.209**
SAPS II	−0.033
Sodium	0.086
Potassium	0.032
Age	0.018
Free cortisol	0.127
Copeptin	0.111
Aldosterone	0.028
Creatinine	−0.187
Urea	0.011
Sex	−0.094
R−squared	0.277
Adjusted R−squared	0.199

* p-value is between 0.1 and 0.01, *** p-value is below 0.001, bold shows significant parameters.

Hormone levels below and above the median of the SAPS II score are shown in Table 8.

Table 8. Comparison of medians (Q1-Q3) of hormone levels below and above the median of the SAPS II score.

	Below the Median of SAPS II (n = 62)	**Above the Median of SAPS II (n = 62)**
Free cortisol (nmol/L) ***	**13 (3–60)**	**73 (31–202)**
Copeptin (pg/mL)	663 (434–1028)	714 (503–1740)
Apelin-13 (pg/mL) *	**2878 (854–3489)**	**1261 (618–3153)**
CRH (pg/mL)	204 (87–317)	127 (75–380)
Aldosterone (pg/mL)	131 (69–283)	215 (110–404)

* p-value is between 0.05 and 0.01, *** p-value is below 0.001, bold shows significant parameters.

Hormone levels according to the septic state are shown in Table 9. Free cortisol showed the sharpest difference of the investigated hormones: septic patients had almost six-fold elevation compared to the non-septic population. The CRH and copeptin were also highly elevated. The median of apelin-13 was higher in the septic group than the non-septic group, but due to the high interindividual variability and low number of septic patients, it was not statistically significant. The kidney function was affected by sepsis, and the values of both urea (p = 0.001) and creatinine (p = 0.005) were significantly elevated.

Table 9. Comparison of medians (Q1-Q3) of SAPS II score and hormone levels between the septic and non-septic groups.

	No Sepsis (n = 94)	Sepsis (n = 30)
SAPS II score *	**39 (26–58)**	**49 (37–65)**
Free cortisol (nmol/L) **	**31 (6–93)**	**172 (38–277)**
Copeptin (pg/mL) ***	**650 (439–909)**	**1637 (694–2934)**
Apelin-13 (pg/mL)	1479 (645–3250)	3230 (2229–4013)
CRH (pg/mL) **	**132 (75–279)**	**573 (322–877)**
Aldosterone (pg/mL)	173 (82–341)	128 (67–544)

* p-value is between 0.05 and 0.01, ** p-value is between 0.01 and 0.001, *** p-value is below 0.001, bold shows significant parameters.

SAPS II score and hormonal parameters according to 30-day survival in the non-septic subgroup (N = 94 patients; 76% of the total population) are shown in Table 10.

Table 10. SAPS II score and hormonal parameters as median (Q1-Q3), according to 30-day survival in the non-septic subgroup.

	Survived at Day 30 (n = 64)	Deceased within 30 Days (n = 30)
SAPS II score ***	**35 (23–43)**	**61 (41–69)**
Free cortisol (nmol/L) *	**24 (4–72)**	**36 (21–138)**
Copeptin (pg/mL) *	**542 (414–880)**	**749 (512–890)**
Apelin-13 (pg/mL) *	**2286 (790–3330)**	**818 (574–2732)**
CRH (pg/mL) *	**201 (84–317)**	**89 (74–233)**
Aldosterone (pg/mL)	158 (78–297)	224 (108–415)

* p-value is between 0.05 and 0.01, *** p-value is below 0.001, bold shows significant parameters.

Cox Regression Analysis

Free cortisol and apelin-13 were significant independent predictors of mortality of the investigated hormonal parameters. In the whole population, free cortisol was the strongest predictor, while in the non-septic subgroup, apelin-13 became a stronger predictor of mortality than free cortisol (Table 11.). Survival function at the mean of apelin-13 and CRH in the whole population and according to the presence of sepsis is demonstrated in Supplementary Figure S1. No difference in the survival of those patients, whose apelin-13 and CRH levels were below and above the mean, was found except in the septic patient group where the apelin-13 level was significantly higher in non-survivors. Interestingly, an opposite trend was found in non-septic patients.

Table 11. Multivariate Cox regression analysis of overall survival according to hormonal parameters.

Investigated Parameters	Chi-Square in the Whole Population	Chi-Square in the Nonseptic Group
Free cortisol	**4.69** *	4.08
Apelin-13	**3.33** *	**3.20** *
Copeptin	0.01	0.70
CRH	0.09	0.02
Aldosterone	0.79	1.23
Number of observations	124	94

* p-value is between 0.1 and 0.01, bold shows significant parameters.

4. Discussion

Our current study investigated various hypothalamic and adrenal hormones in a population with a mixed critical illness. According to previous works, the free cortisol and copeptin serum concentrations were significantly higher among non-survivors [5,10,11]. Apelin-13 showed an opposite change, with a significantly decreased level in more severe cases represented above the median SAPS II score. The apelin-13 serum level was significantly lower in the subgroups of higher SAPS II severity scores and non-septic non-survivors. Furthermore, apelin-13 concentration significantly negatively correlated with the SAPS II severity score during univariate and multivariate analyses. Cox regression analysis was used to find the independent predictors of survival. Several models containing the routinely investigated laboratory parameters were used. Based on the sequence of removal, these models are appropriate to determine the importance of the individual factors. Of the investigated humoral parameters, apelin-13, in addition to free cortisol, was an independent determinant of survival. At the same time, copeptin, CRH, and aldosterone were dropped out. However, it is important to mention that the survival curves according to the mean of apelin-13 showed an opposite tendency in septic and non-septic patients. In the septic subgroup, highly elevated apelin-13 was related to poorer survival. In the non-septic group, a poorer survival trend was found by Cox regression analysis in those who had lower apelin-13 levels. Therefore, the regulation of apelin-13 in septic and non-septic critically ill conditions may be different and further studies are required to recruit a higher number of septic patients.

Apelin has been found to be an essential biomarker for heart failure [29]. Moreover, plasma apelin concentrations were depressed early after myocardial infarction, but did not correlate with the left ventricular function parameters [30]. In patients with ST-segment elevation myocardial infarction, the major adverse cardiovascular events were significantly more common in the low apelin group than in the high apelin group [31]. However, to the best of our knowledge, ours is the first study demonstrating a negative association between serum apelin-13 concentrations and the severity of critical illness. Contrary to our findings, Lesur et al. found no evidence of correlations between apelin-12, another apelin isoform, and either severity or outcome in critically ill patients exhibiting systemic inflammatory response syndrome [32]. These discrepant results may be due to the different patient populations (septic or non-septic; for example after myocardial infarction) and/or the different investigated apelin isoforms. For example, in the study of Lesur et al., even copeptin was not significantly higher in critically ill patients than in normal volunteers, despite the numerous concordant well-demonstrated results showing marked elevation of copeptin in these patients [7–9,12,33,34].

The biological efficacy of the apelin system is compromised under some environmental pressure. For example, in human sepsis, endogenous apelinergic levels rise early, and specific enzymatic breakdown activities potentially threaten endogenous apelin system reactivity and negatively impact the outcome [35]. Furthermore, the short-term exogenous apelin-13 infusion helps stabilize cardiorenal functions in ovine septic shock; however, this ability might be impaired by specific enzymatic systems triggered during the early course of human sepsis [35].

To the best of our knowledge, this is also the first study to investigate serum CRH concentrations in a critically ill population. Compared to the reference range, serum CRH was highly elevated in these patients, especially in septic ones, and positively correlated with apelin-13 and copeptin; however, interestingly, not with free cortisol. Furthermore, serum CRH has strongly determined apelin-13 levels among these patients. Moreover, similar to the apelin-13, serum CRH was significantly higher in surviving non-septic patients than in those deceased within 30 days. No previous human data are available regarding the strong correlation between CRH and apelin-13, so the explanation is only hypothetical; extreme stress reaction may be responsible for the elevation of both hormones. Our knowledge about the degradation of CRH and apelin-13 is also incomplete. It is well known that the elevation of cortisol levels in critical illness is partly due to the decreased degradation process [4]. It may explain the lack of correlation between CRH and free cortisol. Contrary to apelin-13 levels, CRH was not a significant parameter in the Cox regression analysis of survival.

Although both apelin-13 and CRH are basically expressed in the hypothalamus, their serum concentrations are determined by other sources and have weak relationships with their local effects on the central nervous system [21]. More complex regulation and stimulating factors can be presumed in these hormonal systems.

It has been previously demonstrated that the stress response of early admitted ICU patients is different in septic vs. non-septic conditions [32]. The septic subgroup of our patients had a more severe illness with a higher SAPS II score. Moreover, the level of the investigated hormones, except aldosterone, was higher among them than in non-septic patients, although the difference in the case of apelin-13 did not reach significance.

Serum aldosterone correlated to the free cortisol and SAPS II severity score; otherwise, aldosterone did not show any remarkable connections with the other parameters.

Many attempts have been made to improve survival in critical illness and to determine a more accurate prognostic score. There is a lack of convincing disease-modifying therapy in multi-organ failure. The investigation of the role of apelin-13 may have three benefits: to better understand the pathophysiological process and the complex regulation of this hormonal system, to find a prognostic marker that may be simpler than the currently used prognostic scores (which contains seventeen parameters), and to identify a potential therapeutic target.

The apelin system is obviously modified by critical illness, and these changes seem to be different in septic and non-septic conditions. In sepsis, the apelin-13 is extremely high; survival is better in those patients who have below the mean apelin-13 level, therefore, in this patient population, the elevation of apelin-13 may be a marker of severity, and a further increase of apelin-13 by the administration of exogenous apelin-13 is questionable. In the non-septic patient population, only apelin-13 remained a significant determinant of survival in multivariate Cox regression analysis. In non-septic patients with circulatory failure and low apelin-13 levels, exogenous apelin-13 administration might improve the prognosis.

Limitations

There may be some possible limitations of the study. The sample size of the investigated population was relatively limited. This may explain why the serum apelin-13 elevation did not reach significance in the patients that survived compared to the non-survived subgroup of the whole population. However, the study's limited size does not seem to influence our basic observations. Furthermore, no normal controls were included. However, this might not affect the observations related to the potential prognostic roles of the evaluated hormonal parameters at admission to the ICU. Moreover, other relevant humoral factors with potential significance, e.g., ACTH, could have also been tested that might have revealed other relevant hormonal interrelations.

5. Conclusions

Serum apelin-13 showed a decreased level in more severe cases of the mixed population with a critical illness. It appears that there is a considerable difference between the septic and non-septic populations with respect to apelin-13 levels. Moreover, the type of vital organ dysfunction markedly influenced not just the apelin-13 but all the other investigated hormones. The concentrations of apelin-13 and CRH had a strong positive correlation and both hormone levels were significantly higher in surviving non-septic patients. In the multivariate Cox regression analysis of the investigated hormonal parameters, apelin-13 and free cortisol were independent determinants of survival, while copeptin, CRH, or aldosterone were not.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm12144801/s1, Supplementary Figure S1. Survival function at the mean of Apelin-13 and CRH in the whole population and according to the presence of sepsis.

Author Contributions: M.G., E.M., and L.B. designed the study, analyzed data, and wrote the manuscript. M.G. performed the statistical analysis. G.P.-D. participated in the evaluation of laboratory results. K.M., G.B., and P.K. organized the recruitment and performed the measurements at admission. C.K. carried out ELISA tests, G.M. performed the free cortisol measurements. T.K. supervised the laboratory investigations. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Written informed consent was obtained from the participants or the participants' parent/legal guardian/next of kin to participate in the study.

Data Availability Statement: The data supporting this study's findings are available from the corresponding author, László Bajnok, upon reasonable request.

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