

Prognostic role of copeptin and ficolin-3 in heart failure: prospective cohort study with five year follow up

PhD thesis

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Introduction

Pathophysiology and prevalence of heart failure

Chronic heart failure (HF) is a common disease, reducing the quality of life and life expectancy. Its prevalence is 1-2%, but it reaches 10% above the age of 70. Heart failure with reduced ejection fraction (HFrEF) is the term used for heart failure, when it is caused by reduced left ventricular ejection fraction. Most common causes of HFrEF are ischemic heart disease, dilated cardiomyopathy, toxic effects of alcohol consumption, viral myocarditis, chemotherapy and genetic disorders, but it is often idiopathic.

Dysfunction, damage and death of the heart muscle cells are the primary causes of the HFrEF. Adaptive mechanisms are activated, which are transmitted mainly by hormonal and neurohormonal systems. The renin-angiotensin-aldosterone system, the endothelin system, the sympathetic nervous systems are activated. The secretion of vasopressin is increased. However these mechanisms have adverse effects and further worsen the symptoms of HF, the function of other organs, and lead to edema and elevated risk of arrhythmias. The extracardiac changes affect the kidneys, bone marrow, liver, brain, lungs and GI tract. Through congestion, hypoxia, apoptosis, malnutrition, decreased appetite, and activation of inflammatory processes these changes lead to the clinical symptoms and signs: anemia, cachexia, liver failure, fluid retention.

Prognosis and prognostic markers of heart failure

The learning of the prognosis of diseases is important, because it helps to prepare for the course of the disease and guides the therapeutic interventions. The five year mortality of HF is high, reaches 50-60%. The well known prognostic factors are the age, severity of HF, the exercise capacity, the left ventricular ejection fraction, presence of accompanying diseases. Nowadays the use of B-type natriuretic peptide (BNP) is part of the routine clinical practice. BNP, which is mainly produced in the stretching left ventricle, has well confirmed prognostic and diagnostic value. However there are numerous pathological pathways being activated in HF, which characterize different aspects of the disease.

Parameters, reflecting different aspects of HF, can be studied in combination for the better estimation of a patient's prognosis. This is the multimarker strategy. There are several multimarker prognostic score systems in HF: The metabolic, hemodynamic and functional (MHF) staging, the Heart failure Survival Score System (HFSS) and the Seattle Heart Failure Model, used since 2006.

Vasopressin

Vasopressin (AVP) is a 9 amino acid hormone with antidiuretic and vasoconstrictor effects, produced in supraoptic and paraventricular nuclei of the hypothalamus. It is secreted from the neurohypophysis to the circulation. AVP is produced by cleavage from the prohormone, neurophysin II and copeptin is produced in equimolar amount.

Copeptin is the C-terminal of the proAVP; it is a 39 amino acid glycopeptid. AVP production is increased mainly by raising serum osmolarity under physiological circumstances. The other stimulus for AVP production is the decrease of circulating blood volume. Increased production stimulates water reabsorption in the kidney, decreases serum osmolarity, elevates blood volume, increases vascular tone, and stimulates cell proliferation. Effects are mediated through specific receptors, the V2 receptors stimulates water retention by activation of aquaporin-2 channels in the collecting duct in the kidney. Due to the small size and fast degradation of AVP, measurement of its serum level by sandwich immunoassay is not convenient. This was the cause of invention of new methods for the measurement of copeptin, which is produced in equimolar quantity compared to AVP. Morgenthaler and his colleagues introduced the new method in 2006.

Vasopressin in heart failure

The level of copeptin, reflecting AVP activity is increased in HF. It suggests the significant change of AVP regulation in HF in contrast to physiological conditions, because hyponatraemia, hypoosmolarity is common, and often severe finding in HF, but AVP level is still high. High AVP and copeptin levels are interpreted in this setting by the action of other AVP elevating mechanisms, the so called non-osmotic stimuli. According to the widespread opinion, the most important non-osmotic stimulus is the hypotension in HF. It is plausible that the short term effects of AVP may be useful in HF, but in a long term, the systemic

vasoconstriction, the increased water retention and edema, the secondary hyponatraemia have adverse sequelae. The first publications were inconsistent regarding the role of AVP in HF, however it was probably caused by the difficulty of measuring AVP levels. The first data on the prognostic role of copeptin in severe HF were published in 2006, and later it was proved in other studies. The follow up period of these studies were in between a few month and one-two years. The additive prognostic information of copeptin upon the clinical data, laboratory results and BNP was not clear. Because copeptin and BNP mirrors different aspects and pathophysiological pathways of HF, one would suppose, that theirs prognostic role is independent and may be additive.

Heart failure and inflammation

Investigations in the past decades unfolded a complex connection between HF and inflammation. Elevated levels of tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6), interleukin-18 (IL-18), soluble TNF receptors, soluble IL-6 receptor and CRP have been described in HF, in the past 25 years. There prognostic significance was revealed. Our research group confirmed that inflammatory molecules, as heat shock protein 70 (HSP70), complement anaphylatoxin C3a, E selectin are related to HF, and have prognostic significance. There are a few hypotheses on the possible mechanisms between inflammation and HF. According to endotoxin hypothesis lipopolysacharids crosses the intestinal barrier in the congested bowels and then activate immune reactions. It is also known, that inflammatory cytokines have direct cardiotoxic effects. Neurohormonal activation directly causes the activation of inflammatory processes. Numerous human and animal studies revealed that in physiological conditions IL-6 induces the secretion of AVP. It is also known, that myocardial damage activates the immune system. The first step of this activation is the modulation of the toll-like receptor system and complement system, both being part of the innate immune system.

Complement system and its role in heart failure

Complement system is part of the innate immune system, consisting more than 40 plasma and regulator proteins, cell surface receptors. The majority of them is inactive, and

becomes activated by proteolysis in a cascade-like fashion. Their main roles are: activation of phagocytosis, lysis of cells and pathogens, opsonisation, activation of adaptive immune system, stimulation of B cell. Activation of complement system may take place on the surface of pathogens, damaged cells and foreign surfaces. The process of activation has three distinct ways. The classical pathway is induced by IgG or IgM antibodies; the alternative pathway is activated directly on different surfaces without immunoglobulin. The lectin pathway is activated on pathogen surfaces, or by damaged, apoptotic cell surface patterns. The proteins of the lectin pathway, the mannose binding lectin (MBL), the collectin (CL-K1) and the ficolins bind to the surface, thus activating the complement system. There is no need for the presence of immunoglobulins. Finally both three pathways lead to the activation of C3 and C5, and then to the production of the terminal C5b-9 complement complex (TCC). When this protein is bound to cell surface (called membrane attack protein, MAC) it leads to the lysis of cells or bacteria. In soluble form it activates the production of cellular inflammatory mediators. The complement system is concerned to be a system to maintain homeostasis and treat tissue damage, in case of uncontrolled, abnormal activation it may have pathogenic role, as it is the case in sepsis and autoimmune diseases.

The connection between HF and the complement system is less understood. First human studies were published in 2001, and contained only a few dozen participants. Higher levels of TCC were measured in HF, and it correlated with disease severity and higher prevalence of adverse events. The level of C1rs-C1inhibitor complex, which is a marker of the activation of the classical pathway, the level of C3bBbP, which is marker of the activation of the alternative pathway, and the level of C3bc, marker of the common pathway were all elevated in chronic HF. Complement activation was linked to the heart, because the concentration of these proteins were higher in the coronary sinus, then in the pulmonary veins, but the concentration of TCC was lower in the coronary sinus. Our research group also confirmed the activation and prognostic role of complement system in HF, when we proved, that level of C3a in HF is higher and it has a prognostic role. C3a plays a central role in the activation pathway of the complement system. However, the role of the lectin pathway in HF has not been investigated so far.

Heart failure and renal function

Chronic HF is often complicated with renal failure (RI), which worsens the prognosis. RI is independent predictor of mortality in HF. The following mechanisms can be in the

background of decreasing glomerular filtration rate (GFR) in HF: decreasing renal perfusion; mesangial contraction due to increased sympathetic activation; decreasing hydrostatic pressure in the glomerular capillaries due to hypovolaemia, hypotension and use of RAAS inhibitors; elevated hydrostatic pressure in postcapillaries and in Bowman capsule, due to elevated central venous pressure, elevated abdominal pressure, presence of other chronic diseases worsening renal function, which are often complicated with HF, such as diabetes mellitus. Although both creatinine and urea nitrogen (UN) are thought to be markers of renal function, several studies revealed, that UN or the UN/creatinine ratio are better prognostic markers in HF than creatinine or GFR. This new observation can be explained by the differences in between the regulation and excretion of UN and creatinine. Creatinine is filtered in the glomeruli and then distally is not reabsorbed; UN is partially reabsorbed after the filtration in the glomeruli. The reabsorption of UN is dependent on its concentration, but partially it is transporter mediated, specific process. The expression of the urea transporter proteins is regulated by AVP and corticosteroids. AVP enhances the reabsorption of UN. This regulation may explain the very good prognostic role of UN in HF. Based on the above mentioned relationship and the connection between AVP and UN many authors raised the possibility, that UN, or UN/creatinine could be a marker of vasopressin activation. However has not been proved yet in HF.

Objectives

In a prospective study of nearly two hundred patients with HFrEF with five year follow up period, we wanted to answer the following questions and wanted to test out hypotheses:

1, **We planned to examine the long term prognostic value of copeptin in HFpEF. We assumed, that copeptin has an additive prognostic value in HFpEF when compared to BNP and clinical parameters.**

2, **We wanted to examine, whether AVP activity correlates to inflammatory parameters, IL-6, creatinine, GFR and UN. We wanted to analyze the possible relation between these parameters by multivariable tests. We also aimed to examine, what is the relative contribution of these parameters in the determination of copeptin levels.**

3, We wanted to examine the connection between HF and three, high concentration serum protein of the lectin pathway of the complement system. We hypothesized, that the serum level of ficolins and MBL is reduced, due to its consumption. We thought, that this reduction of their serum levels is related to disease severity, neurohormonal and hemodynamic parameters and prognosis of HF.

Methods

Patients

Consecutive patients were recruited to the prospective study in the 3rd Department of Internal Medicine, Semmelweis University both in-, and patient cardiology departments. Inclusion criteria included chronic heart failure, signed informed consent and left ventricular ejection fraction below 45%.

The study was conducted with the permission of the Institutional Ethics Committee of Semmelweis University in Budapest. Malignant diseases, fever, clinical signs of an ongoing infection were the exclusion criteria. There were recruited 196 patients between February 2005 and March of 2007. Functional capacity and clinical data were evaluated by experienced specialist. Data were collected on medical history and the result of bed side examination.

The patients were contacted at year 5 after inclusion and clinical status was registered. Information on mortality (with specific cause of death) was collected from the hospital database, medical records or from family members. Date of censoration was exactly five years after inclusion. Healthy controls (n= 100, 54 women; mean age 36 years) were recruited in an outpatient department in Budapest providing regular health checkup for healthy employees on a mandatory basis.

Laboratory methods

Blood was drawn from peripheral veins, after at least 6 hours of fasting. For routine laboratory tests blood was drawn to native collecting tubes, or tubes containing EDTA or

citrate. Other native tube, tubes with EDTA and citrate were used for preparing serum and plasma aliquots, which were frozen at -70 °C till later use.

Laboratory tests of the complement system

Measurement of copeptin was performed by the inventor of the method, BRAHMS Ltd. (Hennigsdorf, Germany) with a method based on sandwich immun-luminometry. Two types of polyclonal antibodies were used, attaching to the C-terminal of copeptin. One of the antibodies was bound to polystyrene tubes, the other was labeled for the measurement of chemiluminescence. The lower detection limit is 1.7 pmol/L. The intra-assay coefficient of variation is less than 20%.

The plasma concentrations of the MBL, ficolin-2 and ficolin-3 were determined by established ELISA-based methods. Microtiter plates were coated with either monoclonal anti-MBL antibody or monoclonal anti-ficolin-2 antibody or monoclonal anti-ficolin-3 antibody in phosphate buffered saline (PBS) overnight at 4°C. Samples diluted 1:25 and 1:400 for MBL, 1:50 for ficolin-2 or 1:640 for ficolin-3 in sample buffer (PBS-T with 1% mouse serum and bovine serum) were added in triplets to washed wells and incubated for 3 hours at 37°C. MBL was detected with biotinylated monoclonal anti-MBL antibody, ficolin-2 was detected with biotinylated monoclonal anti-ficolin-2 antibody and ficolin-3 was detected with biotinylated monoclonal anti-ficolin-3 antibody by incubation overnight at 4°C. Washed wells were incubated for 1 hour at 37°C with HRP-conjugated streptavidin. Plates were developed for 15 minutes with OPD (o-phenylenediamine) substrate solution and stopped by adding 1M H₂SO₄. The optical density was measured at 490 nm. As standard was used a serum pool which has been calibrated towards recombinant MBL, ficolin-2 and ficolin-3 and thus contain known concentrations of the different proteins. Lower limit of detection in these assays is 20 ng/ml of MBL, 5 ng/ml of ficolin-2 and 1 ng/ml of ficolin-3, respectively. The inter-assay coefficient of variation (CV) is 8.0%, 7.1% and 4.7% and the intra-assay CV 5%, 4.3% and 3.9% for the MBL, ficolin-2 and ficolin-3 assays, respectively. Measurement was performed by Peter Garred and his colleagues in Copenhagen, Denmark.

Laboratory tests of NT-proBNP, TNF- α and IL-6

Levels of NTproBNP (Biomedica ELISA kit (Cat. No BI20852) were measured according to the manufacturer's instructions. Standard and specific laboratory parameters were measured by Roche Integra 800 or by Cell-Dyn 3500 hematology analyzer. Glomerular filtration rate (GFR) was calculated in SI units with help of MDRD equation, according to the National Kidney Foundation Inc. Levels of serum interleukin-6 (IL-6) (R&D Systems High Sensitivity ELISA kit serum (Cat No. HS600B)), tumor-necrosis factor-alpha (TNF- α) (R&D System high sensitivity ELISA kit (Cat No. HSTA00C)) were measured according to the manufacturer's instructions.

Statistical analysis

For descriptive purposes the values of each measurement are given as median and 25th–75th percentile, or as numbers (percent), since most of the variables were not normally distributed. Nonparametric tests were used for group comparisons. Nonparametric tests were used for group comparisons in case of clinical variables; continuous variables between two groups were compared with Mann-Whitney U test and with more groups with Kruskal-Wallis test, whereas categorical variables were compared with Pearson's chi square test. Correlation analysis was done by the Pearson's method on log-transformed variables. Multiple linear regression models with stepwise forward selection process were also applied for continuous variables. Kaplan-Meier analysis and log-rank test was used to analyze survival across different strata. Survival times were measured from inclusion into the study up to the endpoint (all-cause mortality), or until the end of follow-up. Different variables were fitted to univariate and multivariate Cox proportional hazard models to assess their effect on survival. Age was analyzed as time-dependent covariate in the multivariate models. Receiver operator characteristics (ROC) analysis was applied to determine the best cut-point for given continuous variables to predict clinical events. Statistical analyses were carried out using the software STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA), SPSS 13.01 (Apache Software Foundation, USA). A p value below 0.05 was used as border for significance through the study.

Results

Results regarding copeptin

We could measure copeptin level in 195 patients (74% male, median age 69.5 (59.3-77.3) years) out of the 196. 110 patients (56%) died during the follow up period. In a univariate model there was significant difference in numerous echocardiographic, laboratory and clinical parameters between those who died and survived

Survival rates are shown in figure 1.: patients with low copeptin level (<10.2 pmol/l) had a five year mortality rate of 0.828, with medium copeptin levels 0.531, and patients with the highest copeptin level(>22.6 pmol/l) had a mortality rate of 0.328 (p<0.001, log rank test).

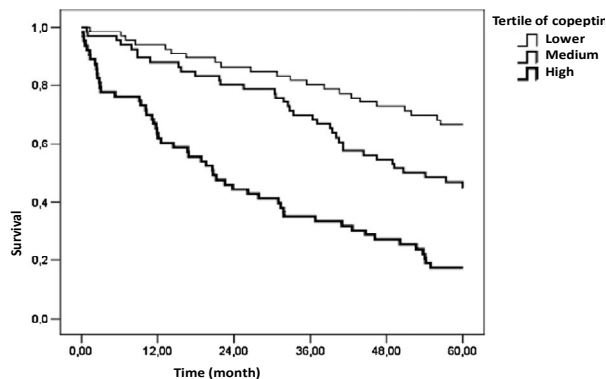


Figure 1. Kaplan-Meier analysis. Survival rates in patients stratified by tertiles of copeptin levels. Log rank test p<0,001. Border for tertiles of copeptin: 33%: 10.2, 66%: 22.6 pmol/L

As the univariate analysis (Table 1) identified copeptin (HR 2.168; 95% CI 1.740-2.700) and several additional variables as predictors of mortality, multivariable models were applied.

Table 1. Final multivariable Cox model for the prediction of death.

Variables	HR	Lower and upper 95% CI for HR		P
NYHA I (reference)	1.000			
NYHA II	2.506	1.097	5.727	0.029
NYHA III	3.312	1.454	7.546	0.004
NYHA IV	3.295	1.221	8.892	0.019
Prior MI	1.495	1.013	2.205	0.043
Urea nitrogen	1.268	1.013	1.589	0.038
GFR (1-SD decrease)	1.421	1.151	1.752	0.001
RDW (1-SD increase)	1.381	1.148	1.661	0.001
NT-proBNP (1-SD increase)	1.368	1.157	1.618	<0.001
Copeptin (1-SD increase)	1.597	1.189	2.146	0.002
Hospitalization due to heart failure in the preceding two years	1.545	1.047	2.279	0.028

Abbreviations: see in text

The final, multivariable Cox survival model identified a number of independent predictors of death. These included higher NYHA functional class, previous MI, at least one hospitalization for worsening HF (within the two years before inclusion into the study), elevated blood urea nitrogen, increased red blood cell distribution width, higher NTproBNP- and copeptin concentrations, and decreased GFR (Table 1).

When a multivariable predictive model was constructed from clinical variables (NYHA class, previous MI, hospitalization due to HF in previous two years, GFR, RDW, BUN), addition of BNP increased the chi-square value by 12.639 ($p < 0.0001$). When copeptin was added to the NT-proBNP adjusted model, a further increase of chi-square value by 7.027 was observed ($p = 0.008$). These results indicate that both BNP and copeptin are significant and independent predictors of mortality, when adjusted for baseline clinical variables.

Association between copeptin concentration and clinical-, laboratory and echocardiographic parameters

Patients were stratified by tertiles of copeptin. Higher copeptin levels were positively correlated with age, NT-proBNP, creatinine, UN, IL-6, TNF- α , CRP and IVC. Hemoglobin, albumin, cholesterol and GFR correlated inversely with copeptin.

A multivariable linear regression model was build to quantify the relative contribution of these parameters to the copeptin level. The model included those variables, which showed correlation with copeptin level (age, NT-proBNP, GFR, UN, hemoglobin, albumin, cholesterol, IVC, IL-6, TNF- α , CRP). According to this model (Table 2) UN and IL-6 shows the strongest correlation with copeptin.

Table 2. Multivariable linear regression model for the examination of copeptin and different clinical and laboratory parameters.

Variable	b	Standard error	T	p value
	0,216729	0,464524	0,46656	0,641613
urea nitrogen	0,748603	0,139620	5,36172	<0,000001
IL-6	0,213317	0,082924	2,57243	0,011249
GFR	-0,298423	0,156360	-1,90856	0,058575
CRP	0,082080	0,052273	1,57022	0,118852
IVC	0,266996	0,255518	1,04492	0,298044
NT-proBNP	0,054652	0,052812	1,03484	0,302710

Role of the lectin pathway of the complement system in the pathophysiology of heart failure.

We could measure the level of components of the complement system in 190 patients. No significant differences for MBL or ficolin-2 concentrations between patients and controls, or across NYHA classes, were observed. However, the patients in both cohorts had markedly lower levels of ficolin-3 as compared to healthy controls ($p < 0.001$), with gradually decreasing levels according to disease severity (Figure 2). According to the results of Dunnett's post test, ficolin-3 levels were significantly lower in patients with NYHA class IV ($p < 0.01$) and class III ($p < 0.01$), as compared to class I patients.

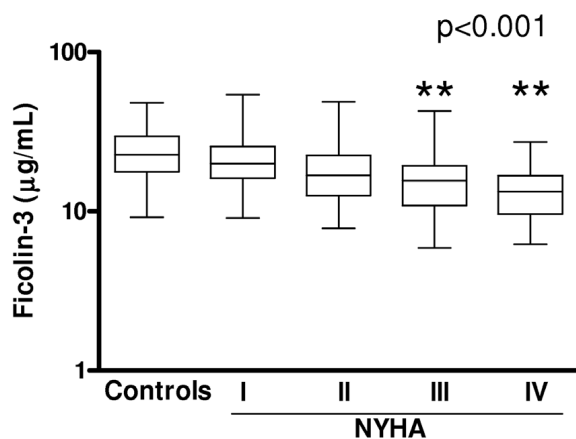


Figure 2
Association ficolin-3 levels with severity of CHF. P values of non-parametric Kruskal-Wallis test are indicated across NYHA class groups, stars indicate results of Dunnett's post hoc test (* $p < 0.05$, ** $p < 0.01$; as compared to NYHA I. Medians, 25 and 75 percentiles and ranges are indicated on logarithmic scales.

Variables in connection with ficolin-3

To find the best cut-off level between low and high ficolin-3 levels, a receiver operating characteristic (ROC) analysis of the ficolin-3 concentration in relation to 5 year survival was performed. The optimal ficolin-3 cut-off level was found to be 15.0 mg/ml. Using this cut-off level it was revealed that a low ficolin-3 level was associated with decreased body mass index (BMI, $p < 0.001$), low total cholesterol ($p < 0.001$) and increased NT-proBNP ($p < 0.001$). No relationship between age or gender, and ficolin-3 levels was observed. Patients with low ficolin-3 levels were further characterized by low C3 levels ($r = 0.417$, $p < 0.001$). The plasma levels of the C3 activation marker C3a inversely correlated with ficolin-3 ($r = -0.198$, $p = 0.008$). These associations between ficolin-3 and NT-proBNP as well as C3 remained significant after multivariable adjustment.

Table 3. Multiple linear regression analysis between a ficolin-3 and clinical-, laboratory parameters.

Variable	Regression coefficient	Standard error	T	p
NT-proBNP	-0.186	0.025	-7.337	0.000
Cholesterol	0.215	0.099	2.174	0.031
Hypertension	0.068	0.023	2.946	0.004
Complement C3a	-0.119	0.037	-3.232	0.001
Complement C3	0.326	0.141	2.309	0.022

Prediction of 5-year Mortality in chronic heart failure by ficolin-3

107 patients died out of 190 (56%) in five years. Univariate Kaplan-Meier survival curves for patient groups with low or high ficolin-3 levels in the two cohorts (grouped by the result of ROC analysis) show an association between low (<15.0 mg/ml) baseline ficolin-3 level and increased all-cause mortality (log rank test, $p < 0.001$) (figure 3.)

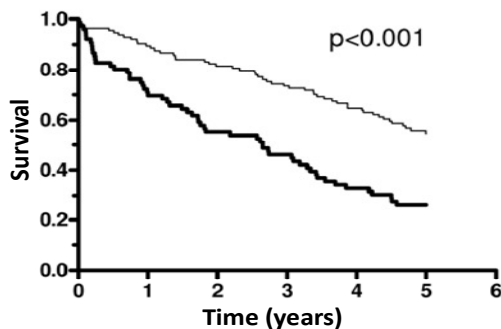


Figure 3. Kaplan-Meier plot of baseline ficolin-3 levels (<15.0 mg/ml, thick line and >15.0 mg/ml, thin line) and long term survival (all-cause mortality) in patients with CHF. P indicates log-rank test.

Ficolin-3 levels showed significant prediction of mortality in the univariate Cox regression analysis (HR 1.709 and 1.471 per 1-SD decrease of ficolin-3). In multivariable age, NT-proBNP-, and gender adjusted models low baseline ficolin-3 was associated with a significantly increased risk of mortality in both cohorts. Ficolin-3 remained a significant predictor of mortality in both cohorts if BMI, diabetes mellitus, hemoglobin, or creatinine were added to the model (table 4.).

Table 4. Results of multivariable Cox proportional-hazards regression analyzing effects of ficolin-3 for all-cause mortality. Hazard ratio for ficolin-3 shown as standardized hazard ratio (HR per 1 SD decrease).

	HR*	95% CI	Wald χ^2 **	p
Ficolin-3, univariate	1,709	1,317-2,212		<0,001
Ficolin-3, (adjusted for age and sex)	1,669	1,283-2,169	17,55	<0,001
Ficolin-3, model 2 (adjusted for age, sex and NT-proBNP)	1,368	1,052	6,210	0,013
Ficolin-3, model 2 (adjusted for age, sex, NT-proBNP and BMI)	1,346	1,033-1,751	5,438	0,020
Ficolin-3, model 2 (adjusted for age, sex, NT-proBNP and diabetes mellitus)	1,389	1,060-1,818	6,467	0,011
Ficolin-3, model 2 (adjusted for age, sex, NT-proBNP, and creatinine)	1,486	1,124-1,953	8,938	0,003
Ficolin-3, model 2 (adjusted for age, sex, NT-proBNP, and hemoglobin)	1,351	1,037-1,761	5,560	0,018

Conclusions

Prognostic role of copeptin

The long-term, five year prognostic role of copeptin was shown in heart failure with reduced ejection fraction in the study. In a multivariable Cox model copeptin was an independent predictor of death even after adjustment for NT-proBNP and clinical parameters. In prior studies with shorter follow up periods copeptin was a predictor of mortality, but not independent of BNP. In other studies copeptin was an independent predictor only for the combined endpoint of mortality and rehospitalization. The predictive value of copeptin was also weaker in a subpopulation of GISSI trial. The differences between our and GISSI trial's result can be explained by the different study population: the borders of copeptin tertiles were higher in our study population, which suggest, that these patients had more severe heart failure. It is also known, that copeptin levels are not proportional to the severity of heart failure: copeptin elevation is higher in more advanced heart failure, and smaller in less severe patients. The NT-proBNP independent value of copeptin seems to be logical, as copeptin and BNP mirrors different pathophysiological aspects of heart failure. BNP is produced in the ventricular wall; the production is induced by wall stretch. Copeptin is synthesized in the brain, which is induced mainly by non-osmotic triggers in heart failure. The fact, that they mirror different pathological pathways in heart failure, may explain their

independent prognostic value. The rather weak correlation between them ($r=0.32$, $p<0.0001$) is also a marker of their different activation pathway. Altogether, it supports the grounds of multimarker strategy.

Variables correlated to copeptin

Our study also aimed the identification of those hemodynamic variables which influence copeptin levels, we examined left ventricular ejection fraction, systolic and diastolic blood pressure, parameters which describe the right- and left atrial pressures: the vena cava inferior, the mitral E and A wave and deceleration time. **In a multivariable linear regression model IL-6 and UN were the strongest correlates of copeptin, but not the hemodynamic variables and parameters connected to filling pressures.**

Copeptin and urea nitrogen

In the multivariable model UN showed the strongest correlation with copeptin. The serum creatinine level is a marker of glomerular filtration rate; however serum level of UN is influenced by the tubular reabsorption. UT-A and UT-B membrane proteins are responsible for the transporter mediated reabsorption of UN, their activity is increased by copeptin. Recently some publications were based on the hypothesis, that level of UN mirrors the activity of AVP system. In an analysis of three, big, retrospective study, the UN/creatinine ratio was used as a renal function independent marker, which describes AVP activity. Renal failure was a predictor of mortality only in patients with an elevated UN/creatinine ratio. Authors conducted, that renal failure is a predictor of mortality only when it is caused by heart failure, and therefore with high AVP activity, that is shown by elevated UN/creatinine ratio. In other publications, positive effects of treatment with vasopressin antagonists were described in those, who had high UN/creatinine ratio, and therefore high AVP activity was supposed. However it was not shown before, that serum levels of UN and copeptin were really correlated in heart failure.

IL-6 and non osmotic stimuli influencing copeptin levels

The cause of the elevated AVP production in heart failure is not the osmotic stimulus, since hypoosmolarity as observed in heart failure is a suppressor of AVP secretion under normal conditions. Original studies showed that arterial and venous baroreceptors activate AVP production when pressure dropped. According to the generally accepted hypothesis this is the mechanism of the AVP activation in heart failure: low intravascular pressure, hypotension induces AVP production through the activation of these baroreceptors. Our results however do not support this hypothesis: the central venous pressure is not decreased, but increased in heart failure, and we could not prove any correlation between systemic arterial pressure and copeptin level.

We found a strong correlation between copeptin and different parameters of inflammation. One possible connection of inflammation and copeptin is the role of IL-6 in the activation of AVP secretion. This connection is well described and studied in physiological conditions, but was not examined in heart failure. It is known, that endotoxin induces IL-1, IL-6 and CRH production and therefore activates the axis of hypothalamus-pituitary-adrenal cortex. Endotoxin induces the production of anterior, but also posterior pituitary (AVP, oxytocin). IL-6 injection into cerebrospinal space induced AVP production in rat. Intravenous injection of IL-6 induced AVP secretion in humans. Treatment of brain with neutralizing antibodies for IL-6 inhibits antidiuretic effect of endotoxin in rat. It was confirmed, that IL-6 is able to cross blood-brain barrier. Our results suggest that these observations are valid also in human heart failure patients. All heart failure clinicians do experience in the daily practice, that heart failure patients become decompensated often by infections and patients get better parallel with the treatment of bronchitis or pneumonia. This clinical experience may be explained by the above mentioned processes, as IL-6 induces VP production, thus leading to increased water retention, edema, hyponatraemia and oliguria.

Chronic heart failure and the lectin pathway of the complement system

We proved that decreasing ficolin-3 concentrations were associated with disease severity in chronic heart failure patients as assessed by clinical NYHA classification and

also strongly inversely correlated with neurohormonal NT-proBNP assessment. Low ficolin-3 levels were related to high concentrations of C3a levels, combined with decreased C3 concentration, linking ficolin-3 levels with complement activation. We demonstrated that low baseline ficolin-3 levels were associated with 5-year mortality. Our publication on heart failure and the complement system was a result of cooperation with a Norwegian research group. The Norwegian group examined 183 patients with HFrEF in a very similar manner than we did. Their results on ficolin-2, ficolin-3 and MBL are similar to our results. The similar outcome of an impendent study population validates our results, and also augments the value of our observations.

MBL, ficolin-2, ficolin-3 are the primary molecules of the lectin pathway of the complement system. They are inductors of the phagocytosis of apoptotic and damaged cells. MBL, ficolin-2, ficolin-3 join to the damage associated molecular patterns (DAMP), which are modified acetylated proteins accessible for the complement system. They activate the complement system and induce phagocytosis. Cell decay in heart failure by apoptosis, necrosis, autophagia and oncosis, or ischemic cell death. It is known, that concentration of MAC is increased in the failing heart. The concentration of the activated molecules of the complement system are higher -, and MAC's concentration are lower in coronary sinus than in the pulmonary veins. This is in consistence with our observations, that decreased concentrations of ficolin-3 are concomitant with decreased C3 and increased C3a concentrations. It suggests that the origin of the decreased ficolin-3 is consumptional. At the same time, the question arises, whether activation of the complement system is only a marker of cell death, an innocent bystander of myocardial cell damage, or has an inductor role in the progressive myocardial cell death, progression of the heart failure, further evolution of myocardial devastation, and has a major pathognomic role in myocardial cell death. It is also possible, that these two processes run parallel fashion.

This is not only a theoretic question, because the blockade of this system may have therapeutic consequences, it could be a new way of heart failure treatment. In former studies, pexelizumab, a humanized monoclonal antibody that binds C5 of complement system showed controversial results in patients undergoing coronary bypass surgery and acute myocardial infarction. I think, more studies are needed in this field, and this may lead to the invention of newer medications in the poor prognostic heart failure patients.

List of publications

Related to Ph.D. thesis

Pozsonyi Z, Forhecz Z, Gombos T, Karadi I, Janoskuti L, Prohaszka Z : Copeptin (c-terminal pro arginine-vasopressin) is an independent long-term prognostic marker in heart failure with reduced ejection fraction. (2015). Heart Lung Circ Apr;24(4):359-67 IF: 1.172

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