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DISSERTATION

Biomarkers in chronic and worsening heart failure patients: Results from the CIBIS-ELD trial and the MOLITOR trial

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1. Abstract

Abstract (Deutsch)

Einleitung: Mit weltweit rund 26 Millionen Erkrankten entwickelt sich die Herzinsuffizienz (HF) zu einer Epidemie. Biomarker sind effektive Werkzeuge für das HF-Management und für diesen Zweck werden derzeit üblicherweise natriuretische Peptide (NT-proBNP und BNP) verwendet. Bislang ist jedoch wenig über den Verlauf von NT-proBNP-Konzentrationen während der Therapieoptimierung bei Patienten mit Herzinsuffizienz mit reduzierter und erhaltener Ejektionsfraktion bekannt. Während einer Episode einer akuten Dekompensation wird NT-proBNP meist mehrmals gemessen. Die Relevanz der seriellen Messungen in Kombination mit neuen Biomarkern (wie Copeptin, MR-proANP, MR-proADM, Endothelin-1) ist jedoch bisher wenig untersucht. Hochsensitives (hs) C-reaktives Protein (CRP) ist mit unerwünschten Ereignissen bei chronischer HF assoziiert. Die Korrelation dieses Biomarkers zur funktionellen Kapazität bei HF-Patienten ist unbekannt.

Ziele: Folgende Fragen zu Biomarkern bei HF wurden im Rahmen dieser Arbeiten beantwortet:

• Wie verlaufen die NT-proBNP-Konzentrationen bei Patienten mit reduzierter (HFrEF) und erhaltener Ejektionsfraktion (HFpEF) während einer 12-wöchigen Beta-Blocker-Titration?

• Welche Biomarker sind die wichtigsten Prädiktoren für Mortalität/Rehospitalisierung bei akut dekompensierten HF-Patienten, wenn sie seriell gemessen werden?

• Korreliert die Konzentration des hs-CRP mit den Veränderungen der funktionellen Kapazität während einer 12-wöchigen Beta-Blocker-Titration bei HF-Patienten?

Methoden: Die durchgeführten Analysen waren Substudien der CIBIS-ELD Studie und der MOLITOR Studie. CIBIS-ELD war eine doppelblinde, multizentrische Studie bei älteren Patienten mit chronischer HF, die auf eine Therapie mit Bisoprolol oder Carvedilol randomisiert wurden. MOLITOR war eine Studie von 164 hospitalisierten Patienten mit akuter dekompensierter HF. Copeptin, NT-proBNP, MR-proANP, MR-proADM und Endothelin-1 wurden bei Aufnahme, nach 24h, 48h und 72h und danach alle 72 Stunden bei Entlassungs- und Follow-up-Visiten gemessen.

Ergebnisse: Beim Vergleich der NT-proBNP-Konzentrationen zwischen den 626 HFrEF- und den 250 HFpEF-Patienten in CIBIS-ELD wurde festgestellt, dass NT-proBNP in der HFrEF-Population während der 12-wöchigen Beta-Blocker-Titration stabil blieb und in der HFpEF-Population leicht anstieg. Der Unterschied der NT-proBNP-Veränderungen zwischen der HFrEF- und HFpEF-Population war statistisch nicht signifikant (P=0,13). Bei 164 akut dekompensierten HF-Patienten der MOLITOR-Studie war Copeptin bei Aufnahme der beste Prädiktor für die 90-tägige Mortalität/Rehospitalisierung ($\chi 2$=16,63, C-Index=0,724, P<0,001). Die erneute Messung nach 72h erhöhte den prognostischen Wert ($\chi 2$=23,48, C-Index=0,718, P=0,00001). Bei 488 HF-Patienten der CIBIS-ELD-Studie fanden wir eine Korrelation zwischen hs-CRP-Veränderungen und Veränderungen der funktionellen Kapazität (6-Minuten-Gehtest; P=0,002).

Schlussfolgerung: Die durchgeführten Analysen leisten einen Beitrag zur aktuellen Biomarkerpraxis bei HF-Patienten. Bei akut dekompensierten HF-Patienten scheint die Messung von Copeptin bei Aufnahme und nach 72 Stunde der beste Prädiktor für die 90-Tage-Mortalität und Rehospitalisierung zu sein. Wir zeigten, dass bei chronischen HF-Patienten Veränderungen des hs-CRP zur Vorhersage der funktionellen Kapazität genutzt werden können.

Abstract (English)

Background: With around 26 million people affected worldwide, heart failure (HF) is growing into an epidemic. Biomarkers are powerful tools for HF management, and currently natriuretic peptides (NT-proBNP and BNP) are routinely used for this purpose. However, little is known about NT-proBNP's trajectories during therapy optimization in different HF populations. During an episode of worsening HF (WHF), NT-proBNP is often repeatedly being measured, but the relevance of its serial measurements in combination with novel biomarkers (copeptin, MR-proADN, MR-proADM, endothelin-1) has not previously been explored. Elevated high sensitivity (hs) C-reactive protein (CRP) is associated with adverse outcomes in chronic HF. Its correlation to functional capacity in HF patients is unknown.

Aim: We sought to answer the following questions concerning biomarkers in HF:

- What is the trajectory of NT-proBNP levels in patients with reduced (HFrEF) and preserved ejection fraction (HFpEF) during a 12-week beta-blocker titration?
- When measured serial, which biomarkers are the best predictors of mortality/rehospitalization in WHF patients?
- Do hs-CRP levels correlate with the changes in functional capacity during a 12-week beta-blocker titration in HF patients?

Methods: The performed analyses were substudies of CIBIS-ELD and MOLITOR trials. CIBIS-ELD was a double blind, multicenter trial in elderly HF patients, randomized to bisoprolol and carvedilol. MOLITOR was a trial of 164 hospitalized patients with WHF. Copeptin, NT-proBNP, MR-proANP, MR-proADM and endothelin-1 were measured on admission, after 24, 48, and 72h, and every 72h thereafter, at discharge and follow-up visits.

Results: While comparing the NT-proBNP levels between the 626 HFrEF and 250 HFpEF patients in CIBIS-ELD, it was noticed that NT-proBNP remained stable in the HFrEF population during the 12-week beta-blocker titration, and slightly increased in the HFpEF population. The difference in NT-proBNP change over 12 weeks between HFrEF and HFpEF patients was not statistically significant (P=0.13). In 164 WHF patients of the MOLITOR trial, copeptin at admission was the best predictor of 90-day mortality/rehospitalization ($\chi 2$=16.63, C-index=0.724, P<0.001). Its remeasurement at 72h increased prognostic value ($\chi 2$=23.48, C-index=0.718, P=0.00001). In 488 HF patients of the CIBIS-ELD trial, we found a correlation between hs-CRP changes and changes in functional capacity (6-minute-walk-test; P=0.002).

Conclusion: The performed analyses make a contribution to the current biomarker practice in HF patients. In WHF patients, copeptin at admission with remeasurement at 72h seems to be the best predictor of 90-day mortality and rehospitalization. We showed that in chronic HF patients, changes of hs-CRP can be used to predict of functional capacity.

2. Background

Heart failure (HF) affects around 26 million people worldwide, and apart from being a serious and debilitating state for the patients, it is a major burden on the health care systems, caregivers and patients' families [1].

Diagnostic and prognostic biomarkers are powerful tools for the management of HF. Currently, natriuretic peptides, and especially B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are routinely used for this purpose. Apart from their importance in diagnosis and prognosis of HF, they are useful in the therapy guidance as well [2]. Natriuretic peptides play an important role in the organism, maintaining homeostasis in the cardiovascular system. In patients with HF, BNP and NT-proBNP are elevated [3]. However, little is known about NT-proBNP's trajectories during therapy optimization in HF with reduced (HFrEF) and preserved ejection fraction (HFpEF).

Worsening heart failure (WHF) is the main reason for hospital admissions worldwide. Problems associated with hospitalization of WHF patients are complex. Acute decompensation points out to the fundamental change in HF progression, and mortality in the years after the hospitalization is higher than in non-hospitalized HF patients. At the same time, patients who were once hospitalized for WHF are likely to be rehospitalized within the next 6 months [4]. Natriuretic peptides are known as important biomarkers for risk stratification of hospitalized WHF patients. It is also known that their decrease over the course of the WHF episode correlates with a better prognosis [5]. However, more biomarkers, such as copeptin, mid-regional pro-adrenomedullin (MR-proADM), CT-proET1 and mid-regional pro-atrial natriuretic peptide (MR-proADN) are emerging and their prognostic value in this patient population is being investigated. Copeptin is a C-terminal fragment of pre-pro-vasopressin and is secreted in equimolar amounts to vasopressin, an important peptide whose values are elevated in HF patients. It has been previously proven that increased levels of vasopressin are associated with the severity of the disease. Vasopressin is not stable and cannot be measured accurately, therefore copeptin is commonly measured instead [6]. Adrenomedullin is another peptide whose levels are elevated in HF patients. Similarly to vasopressin, it is not stable in plasma, therefore mid-regional section of its prohormone (MR-proADM) is used as a biomarker [7]. Endothelin-1 is a vasoconstrictor and pro-fibrotic hormone, and it is very often elevated

in HF patients, as a result of neurohormonal activation. Its stable precursor, CT-pro-endothelin-1 (CT-proET1) is commonly being measured [8]. Atrial natriuretic peptide (ANP) is released from the atria in response to increased intra-atrial pressure and wall stretch, and has diuretic, natriuretic, and vasodilatory effects. Its concentrations cannot be easily measured, which is why the mid-region of its prohormone (MR-proANP) is used as its marker [9]. However, not much is known about the trajectories of these biomarkers during an WHF episode.

Systemic inflammation and elevated levels of circulating inflammatory cytokines in HF patients are gaining more and more research interest lately [10]. Significant amount of data from multiple studies showed increased hs-CRP levels in HF of different etiologies (ischemic heart disease, idiopathic dilated cardiomyopathy, or valvular heart disease) and an association of this increase with an adverse outcome. This suggests that inflammatory activation in HF is independent of the etiology [11]. It has also been shown that CRP predicts mortality in patients with established HF and that higher CRP levels are associated with higher NYHA class and greater severity of HF [12]. CRP levels can be influenced by different cardiovascular drugs and in particular by beta blockers [13] [14].

The six-minute walk test (6MWT) is a submaximal exercise test used for evaluation of functional capacity in HF patients [15]. Improvement of functional capacity is one of the most important patient centered outcome in HF therapy. The correlation of hs-CRP levels to functional capacity in HF patients is still unknown.

3. Aim

The presented work aimed to answer the following three questions:

1. What is the trajectory of NT-proBNP levels in patients with HFrEF and HFpEF during a 12-week beta-blocker titration?
2. When measured serial, which biomarkers are the best predictors of mortality/rehospitalization in worsening HF patients?
3. Do hs-CRP levels correlate with the changes in functional capacity during a 12-week beta-blocker titration in HF patients?

4. Methods

Data used in this work was obtained from two trials performed in the population of HF patients: CIBIS-ELD (The Cardiac Insufficiency Bisoprolol Study in ELDerly) and MOLITOR (Impact of Therapy Optimisation on the Level of Biomarkers in Patients with Acute and Decompensated Chronic Heart Failure).

CIBIS-ELD was an investigator-initiated, phase 3 trial in chronic HF patients, with a main objective to compare the tolerance of bisoprolol and carvedilol in these patients [16]. It was a multicenter trial (55 centers in four countries), 1:1 randomized and double-blind. In order to be eligible for the CIBIS-ELD trial, patients had to be 65 years old or older, and beta-blocker naïve or on 1/4 of the guideline recommended target or equivalent dose at baseline. In total, 883 patients have been enrolled and randomized. Once enrolled in the trial, patients were 1:1 randomized to bisoprolol (10 mg/day target dose) or carvedilol (25 mg b.i.d target dose) [16].

To answer the Aim 1, NT-proBNP was measured in 625 HFrEF and 250 HFpEF patients. To answer the Aim 3, NT-proBNP and hs-CRP were measured and the 6MWT has been done in 488 both HFpEF and HFrEF patients.

Data obtained from the MOLITOR trial was analyzed in order to answer the question presented in the Aim 2. MOLITOR was a prospective biomarker study of hospitalized patients with WHF (N=164), with the primary endpoint being the best time point to predict all-cause death or rehospitalization within 90 days. Main inclusion criteria were presentation of HF signs and symptoms with dyspnoea at rest or minimal exertion (NYHA class III and IV) and pulmonary congestion on physical examination or chest X-ray. In case of suspected acute myocardial infarction, cardiogenic shock, sepsis or active infection requiring i.v. antimicrobial treatment, significant arrhythmias, significant kidney disease with current or planned hemofiltration of dialysis, acute myocarditis, hypertrophic cardiomyopathy, alternative diagnosis that could explain patient's HF symptoms, or age less than 18 years patients were not included in the study [17].

Biomarker measurements

In the CIBIS-ELD trial, NT-proBNP and hs-CRP were measured from blood samples taken at baseline and after 12 weeks of treatment. These samples were taken in standardized conditions by venous puncture after a 20-minute resting period, centrifuged immediately and stored below -80 degrees Celsius. Levels of NT-proBNP and hs-CRP have been afterwards determined using commercially available assays (Elecsys, Roche Diagnostics).

For the measurement of biomarkers in WHF patients, blood samples were taken at admission, after 24, 48, and 72h, and every 72h thereafter, at discharge and at regular follow-up visits. Blood samples were collected in ethylenediaminetetraacetic acid. NT-proBNP (Elecsys 2010 analyzer, Roche Diagnostics, Indianapolis, Indiana), copeptin and MR-proANP (both on the KRYPTOR system) were measured centrally through BRAHMS/Thermo Fisher, Germany. MR-proADM was measured using an automated sandwich chemiluminescence immunoassay on the KRYPTOR system.

Statistics

In the analysis of NT-proBNP levels between HFpEF and HFrEF patients [18], changes from baseline to 12 weeks were assessed in each group by t tests for paired variables and were compared between groups by analysis of covariance with the 12 weeks value as dependent variable, the baseline value as covariate, and ventricular function as the group variable. Analyses were performed using SPSS version 15 software (SPSS Inc., Chicago, Illinois).

In the MOLITOR trial, Student t-test for paired samples on log10-transformed data was used for the assessment of the biomarker level changes, and Spearman rank correlation coefficients for the assessment of the relationship between biomarkers. Primary endpoint of the trial was all-cause mortality and/or hospitalization at 90 days. Cox regression models were created for each biomarker for baseline values for the primary endpoint. Patients were stratified according to tertiles of copeptin and NT-proBNP, and Kaplan-Meier estimates of event-free survival probabilities were computed. In order to compare survival curves between the groups, log-rank tests were performed. One of the questions of the MOLITOR trials was also if there are significant added values of certain follow-up measurements (on top of measurements

obtained at admission): this was tested using the time-dependent Cox model. Significance level of 0.5 was defined for all the tests. For the statistical analyses in the MOLITOR trial, software R 3.2.0 was used.

In order to answer the third question in the presented work [19], four groups of patients were defined based on the hs-CRP level at baseline (low or high) and after 12 weeks (low or high). The cut-off which defined low or high hs-CRP values was set at 0,3 mg/dl, as per American Heart Association and U.S. Center for Disease Control and Prevention guidelines. These guidelines state that if a patient has hs-CRP levels < 0,1 mg/dl the cardiovascular risk is low, if the hs-CRP levels are between 0,1 – 0,3 mg/dl the cardiovascular risk is medium and above 0,3 mg/dl the cardiovascular risk is high. Therefore, four groups have been identified: (1) low hs-CRP at baseline and low at follow-up (low-low), (2) high hs-CRP at baseline and high at follow-up (high-high), (3) low hs-CRP at baseline and high at follow-up (low-high), (4) high hs-CRP at baseline and low at follow-up (high-low). Raw data for NT-proBNP and hs-CRP have been log-transformed prior to analysis, as they follow a log-normal distribution. Differences between baseline and follow-up are computed from raw values. Change in 6MWT was tested by a multifactorial ANOVA with CRP group change, age, gender, NYHA, BMI, blood pressure, baseline walking distance, and CAD as independent variables. Analyzes were performed using R version 3.4.0.

5. Results

1. What is the trajectory of NT-proBNP levels in HFrEF and HFpEF patients during a 12-week beta-blocker titration?

Out of 883 patients from the CIBIS-ELD trial, 876 patients were suitable for this sub-analysis (mean age 72.8 ± 5.5, 38% female; mean LVEF 58.7 ± 8.8% in HFpEF, 34.9 ± 8.0% in HFrEF). More patients were diagnosed with HFrEF (71%). In both HFpEF and HFrEF groups, 51% of patients were randomized to carvedilol, and 49% to bisoprolol. Looking into the two analysed groups, we noticed that HFpEF patients were more frequently female and older, with higher systolic blood pressure and lower resting heart rates at baseline, higher body mass index levels, in lower NYHA functional classes and with more peripheral edema. More of HFpEF patients analysed

in this work were beta-blocker-naïve at the beginning of the study (52% compared to 35% in HFrEF).

Particularly interesting for this work was the difference noticed with concerning the NT-proBNP levels. Patients with HFpEF seem to have significantly lower NT-proBNP levels compared to HFrEF patients. At baseline, in HFpEF patients, the median NT-proBNP levels were 253 (161-529) pg/ml, whereas in HFrEF patients the levels were significantly higher 968 (409-2091) pg/ml (P<0,001). Over the 12 weeks, we have noticed interestingly an increase of NT-proBNP in HFpEF patients. In HFrEF patients, however, NT-proBNP remained stable. The difference in NT-proBNP level change during 12 weeks of beta-blocker therapy between HFrEF and HFpEF patients was not significant (P=0.13). More details are presented in Table 1.

Table 1. NT-proBNP changes from baseline to 12 week of beta-blocker treatment

	HFpEF (N=250)	HFrEF (N=626)	HFpEF vs HFrEF*
Log10 NT-proBNP Mean change (95% CI)	0.05 (0.00 to 0.10) P=0.03	1.(-0.04 to 0.03) P=0.82	0.05 (-0.01 to 0.11) P=0.13

**Adjusted for beta blocker pre-treatment and study drug*

2. When measured serial, which biomarkers are the best predictors of mortality/rehospitalization in worsening HF patients?

The MOLITOR study included 164 WHF patients (mean age 69, 70% male, mean LVEF 34%, 76% in NYHA class III). The mean length of their hospital stays was 7 days, and 8 patients died during the initial hospitalization. Analysis of the data obtained after 90 days showed that 14.1% of patients were rehospitalized and 10.6% of patients died.

All analyzed biomarkers except for MR-proADM decreased significantly from initial hospital admission to discharge (P<0.005). At baseline, all biomarkers except for CT-proET1 were significant univariate outcome predictors: copeptin was the strongest predictor ($\chi 2$=16.63, C-index=0.724, P<0.001), followed by NT-proBNP ($\chi 2$=10.53, C-

index=0.646, P=0.001). NT-proBNP's prognostic performance for 90-day outcome improved with the addition of baseline copeptin (model likelihood ratio (LR) $\chi 2$=17.80, C-index=0.688, P=0.004). Addition of other novel biomarkers, including MR-proADM did not result in the same way.

It was shown that if copeptin is remeasured after 72h from admission, its predictive value increases (copeptin $\chi 2$=23.48, C-index=0.718, P=0.00001). Patients with low levels of copeptin (< 50pmol/L) at admission and at 72h had the best prognosis (Figure 1), whereas the patients whose copeptin levels were persistently elevated during the first 72h after the hospitalization had the worst prognosis at 3 months' follow-up. It was also shown in this analysis that an additional copeptin remeasurement after 48h resulted in added prognostic value (LR $\chi 2$=19.08, added $\chi 2$=5.1, P=0.024).

NT-proBNP showed the highest predictive value when remeasured after 48h ($\chi 2$=14.23, C-index=0.650, P<0.001). Patients with the best prognosis had NT-proBNP levels low at admission and low after 48h. On the other hand, patients with the worst prognosis had low NT-proBNP levels at admission but elevated after 48h (Figure 2).

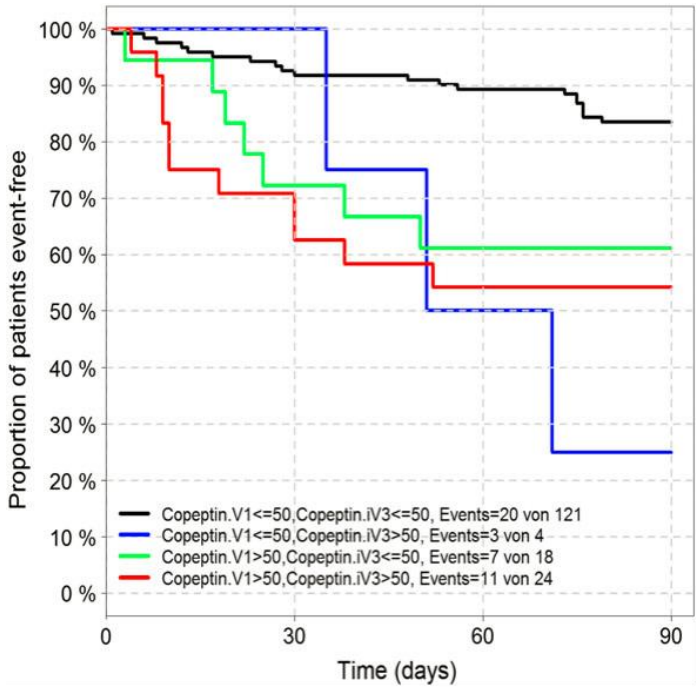

Figure 1. Kaplan-Meier analysis of event-free survival according to the change of the concentration of copeptin 72h after hospitalization (Düngen *et al.*, 2018)

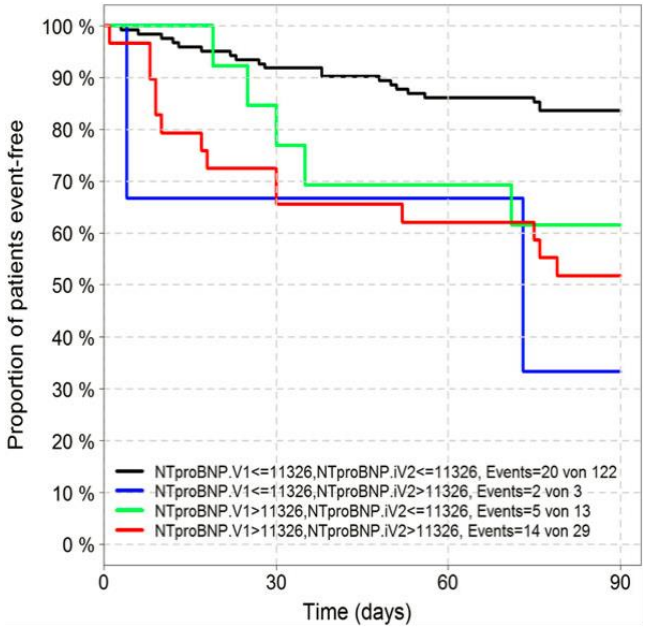

Figure 2. Kaplan-Meier analysis of event free survival according to the change of NT-proBNP concentration 48h after hospitalization (Düngen *et al.*, 2018)

Contrary to copeptin and NT-proBNP, remeasurement of MR-proANP, MR-proADM and CT-proET1 during hospitalization did not provide additional prognostic value.

3. Do hs-CRP levels correlate with the changes in functional capacity during a 12-week beta-blocker titration in HF patients?

For this analysis, patients were divided in four groups based on their hs-CRP levels at baseline and after 12 weeks: (1) low-low, (2) low-high, (3) high-low and (4) high-high. Affiliation to a certain group was determined based on the hs-CRP level at baseline and at follow-up, and the cut-off value was 0.3 mg/dl.

More patients were male (64%) with the mean age of 72.1 ± 5.31 years. Median left ventricular ejection fraction (LVEF) was 40 (33/50) % and the average distance of the 6MWT at baseline was 334 ± 105 m.

Total number of analyzed patients was 488, and 225 were in the low-low group, 132 in the high-high group, 54 in the low-high and 77 in the high-low group. In the low-low group baseline (BL) hs-CRP level was 0.14 (0.08/0.2) mg/dl, and after 12 weeks (follow-up, FU) was 0.13 (0.08/0.19) mg/dl. In the high-low group, BL hs-CRP was 0.53 (0.37/0.89) mg/dl and FU hs-CRP was 0.18 (0.13/0.22) mg/dl. The high-high group expectedly showed the highest hs-CRP levels: 0.66 (0.43/1.13) mg/dl at BL and 0.62

(0.43/1.20) mg/dl at FU. In the low-high group, the hs-CRP levels were 0.16 (0.11/0.24) mg/d at BL and 0.43 (0.40/0.60) mg/dl at FU.

The overall relative change of NT-proBNP from baseline (BL) to after 12 weeks (FU) was Δ % FU-BL NT-proBNP=5 (-28/54). Looking closely to each of the defined groups, we noticed that the biggest decrease of NT-proBNP was seen in the high-low group (Δh->l% FU-BL NT-proBNP= -8 (-42/32)), whereas the biggest increase was in the low-high group (Δl->h % FU-BL NT-proBNP = 30 (-14/88)). The change of NT-proBNP in the low-low was close to the one seen in the overall population (Δl->l % FU-BL NT-proBNP=5 (-28/51)), and the relative change in the high-high group was Δh->h % FU-BL NT-proBNP=2 (-25/56). We also showed that there was a statistically significant difference in the relative NT-proBNP change between the four defined groups (P=0.011, Figure 3).

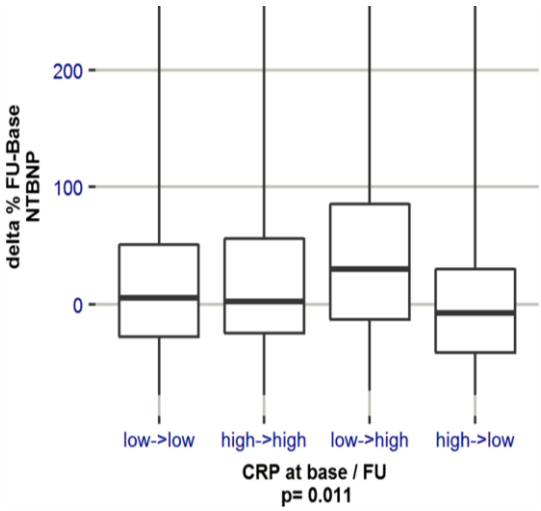

Figure 3. Association between the relative change of NT-proBNP and hs-CRP from baseline to follow-up (Radenovic S *et al.*; 2018)

Looking at the results of the 6MWT, it can be noticed that there was a trend towards an increase of the distance in the overall population: at BL it was 334±105 m and after 12 weeks 348±99 m. In a more detailed look to the results in each group, we showed that the absolute change in 6MWT results from baseline to follow-up was significantly different between the four defined groups (P=0.002, Fig. 4). The biggest increase in 6MWT distance between baseline and follow-up was in the low-low group (Δl->l 6MWT = 24±62 m), and the biggest decrease occurred in the low-high group (Δl->h 6MWT= -

18±90 m). The changes from BL to FU in the high-high and the high-low group were close to the overall change (Δh->h 6MWT=15±69 m and Δh->l 6MWT=14±62 m).

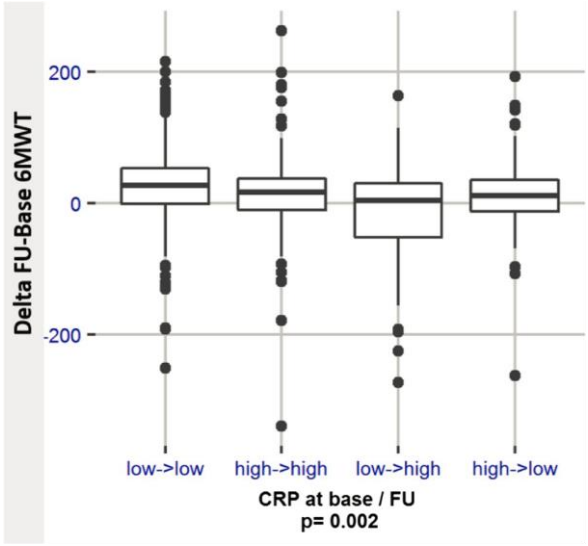

Figure 4. Association between the change of 6MWT and hs-CRP from baseline to follow-up (Radenovic S *et al.*; 2018)

6. Discussion

In the last years, the research of biomarkers in HF has increased significantly. With this work, we aimed to contribute to the identification of prognostic biomarkers in chronic and worsening HF patients.

In the analysis of NT-proBNP level changes in patients with HFpEF vs HFrEF who were randomized to two different beta-blockers for 12 weeks, we found that HFpEF patients had lower NT-proBNP levels at baseline. However, despite NT-proBNP remaining stable in HFrEF patients over 12 weeks, levels in HFpEF increased over this time period. The overall difference in NT-proBNP level change during 12 weeks of beta-blocker therapy between HFrEF and HFpEF patients was not significant.

As pathophysiology of HFrEF seems to be more associated with cardiac stretch [20], it was expected to find higher NT-proBNP values at baseline in this subgroup. Another study exploring different biomarkers in HFpEF vs HFrEF patients found higher NT-proBNP levels in HFrEF patients [2142 (1473-4294) vs. 4202 (2239-7411), P<0.001].

This study has also shown that in the NT-proBNP-guided study arm, NT-proBNP had less prognostic value in HFpEF patients, when compared to HFrEF [21].

The second part of this analysis showed increased NT-proBNP levels in HFpEF over the 12-week beta-blocker titration period, which could indicate a worsening of left ventricular function. This is an interesting finding, because of the ongoing discussion if beta-blocker treatment could benefit the HFpEF patients as well. As an example, the SENIORS study (Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalization in Seniors with Heart Failure) showed that nebivolol reduces the risk of either mortality or cardiovascular hospitalizations in both HFpEF and HFrEF patients [22]. However, the ELANDD study, which explored nebivolol vs placebo in HFpEF patients, also showed a slight increase of NT-proBNP in the nebivolol group during 6 months of treatment. Further, nebivolol was not associated with an improvement in the 6MWT distance [23]. However, in our analysis, no effect on E/E' or LAVI was noticed during the 12-week beta-blocker titration. These results combined could point out to the conclusion that beta-blockers are not helpful in this patient population.

The second analysis presents the largest sample of serial in-hospital measurements of novel biomarkers in WHF patients during hospitalization. Copeptin was shown to be the strongest predictor for all-cause mortality or hospitalization at 90 days. Further, patients with consistently elevated copeptin (remeasured after 72 hours) had the worst prognosis at 3 months follow-up. Some of the previous research points out to the same direction. In the BACH (Biomarkers in Acute Heart Failure) trial, patients with elevated copeptin levels were at higher risk of 90-day mortality, readmissions and emergency department visits [24]. Another study proved that in patients with chronic HF, copeptin levels may predict mortality, independently from clinical variables [25]. Therefore, it seems that the conclusion from the research done so far is that copeptin has prognostic value in HF patients.

Remeasurements of biomarker for monitoring the response to a particular therapy have recently caught much interest. Our research showed that remeasurement of copeptin 72 hours after the initial admission could improve risk stratification. Another study showed, similarly to ours that patients with dyspnea who have high copeptin levels at both admission and discharge have higher risks of death or rehospitalization

at 90 days [26]. However, there are other studies which showed, contrary to our results, that changes in copeptin levels over time do not give any additional prognostic value [27], [28].

As HF is a complex state, it is understandable to search for combination of biomarkers which would together make a better prognostic marker. Our analysis has shown that prognostic performance of copeptin is significantly improved with the addition of baseline NT-proBNP levels. Similar to our research, a previous analysis of 470 elderly HF patients showed that the combination of increased concentration of copeptin and NT-proBNP are related with increased mortality [29].

While answering the third question of this work, we found that the change in 6MWT distance in HF patients treated with beta-blockers is associated to the change in hs-CRP levels from baseline to follow-up. We also showed that the change of hs-CRP from baseline to follow-up is associated with the relative change of NT-proBNP in the same patient population.

Inflammation in cardiovascular disease and especially in HF patients has been gaining more and more interest over the years, as previous research showed that inflammatory biomarker levels are increased in HF regardless of etiology and that their levels correlate with the severity of disease [30], [31]. Recently, there is an increasing amount of clinical data showing that by reducing inflammation, the risk of cardiovascular diseases can be reduced [32].

Some of the medication commonly prescribed to HF patients, such as beta-blockers, renin–angiotensin–aldosterone antagonists and statins seem to decrease the levels of inflammatory markers in HF patients [33]. Studies exploring the anti-inflammatory effects of beta-blockers consistently show a decrease in CRP levels of HF patients [34],[35]. In our research, 57% of patients whose data were analyzed had a decrease of hs-CRP level during the 12 weeks of treatment with beta-blockers. As patients were randomized to a selective (bisoprolol) and a non-selective (carvedilol) beta-blocker, we tested to check if selectivity is an important factor for anti-inflammatory effect of beta-blockers. However, it seems that the selectivity did not play a role, because each

defined group (low-low, low-high, high-low and high-high) had a similar distribution of patients randomized to bisoprolol and carvedilol.

As previously mentioned in this work, natriuretic peptides are the current gold standard for evaluating the disease status and prognosis in HF patients. A large amount of previous data supports NT-proBNP as an important biomarker in cardiovascular diseases [36]. Our analysis showed that during the 12 weeks of beta-blocker treatment, the hs-CRP level changes follow the changes in NT-proBNP: in the high-low group the decrease of NT-proBNP was followed by the decrease of hs-CRP, whereas in the low-high group the increase of both NT-proBNP and hs-CRP was noticed.

Functional capacity is an important patient centered outcome. However, not many studies have explored the correlation between the levels of inflammatory biomarkers and functional capacity. As a part of this analysis, we showed that with the increase of hs-CRP, patients show worse results on the 6MWT. We also showed that in case that hs-CRP remains stable or decreases, the 6MWT results improve. However, it seems that initially low hs-CRP levels at baseline which remain low during the 12 weeks of beta-blocker treatment result in the biggest improvement of 6MWT. In patients whose hs-CRP levels were high at baseline and decreased over time, the 6MWT results were better after 12 weeks, but the improvement was not as big as in the low-low group. This led us to the conclusion that the control of inflammation might be beneficial in all heart failure patients, and especially in those whose hs-CRP levels are above 0,3 mg/dl, because it is connected to a physical improvement of the patient.

7. Conclusions

The performed analyses contribute to the current biomarker practice in HF patients. The slight increase in NT-proBNP levels during beta-blocker titration can point to the conclusion that beta-blockers might not be helpful in the HFpEF population. With the MOLITOR analysis, we have concluded that in worsening HF patients, copeptin at admission with remeasurement at 72h seems to be the best predictor of 90-day mortality and rehospitalization. Further, we showed that in chronic HF patients lowering of hs-CRP relates to an improvement of functional capacity underlining the relevance of inflammation in HF.

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9. Declaration of publications

Sara Radenovic had the following share in the three publications listed below:

Publication 1:

Edelmann F, Musial-Bright L, Gelbrich G, Trippel T, **Radenovic S**, Wachter R, Inkrot S, Loncar G, Tahirovic E, Celic V, Veskovic J, Zdravkovic M, Lainscak M, Apostolović S, Neskovic AN, Pieske B, Düngen HD. Tolerability and Feasibility of Beta-Blocker Titration in HFpEF Versus HFrEF: Insights From the CIBIS-ELD Trial. JACC Heart Fail. 2016 Feb;4(2):140-9.

Contribution: Interpretation of data, co-writing of the manuscript (sections: Methods, Discussion - clinical effects, Perspectives), review of the manuscript

Publication 2:

Düngen HD, Tscholl V, Obradovic D, **Radenovic S**, Matic D, Musial-Bright L, Tahirovic E, Marx A, Inkrot S, Hashemi D, Veskovic J, Apostolovic S, von Heahling S, Doehner W, Cvetinovic N, Lainscak M, Pieske B, Edelmann F, Loncar G, Trippel T. Prognostic performance of serial in-hospital measurements of copeptin and multiple novel biomarkers among patients with worsening heart failure: Results from the MOLITOR study. ESC Heart Fail. 2018 Apr;5(2):288-296. doi: 10.1002/ehf2.12231. Epub 2018 Feb 24.

Contribution: Interpretation of the data, co-writing of the manuscript (sections: Introduction, Results, Discussion), preparation of the figures for the publication, critical review of the manuscript

Publication 3:

Radenovic S, Loncar G, Apostolovic S, Zdravkovic M, Veskovic J, Karlicic V, Tahirovic E, Butler J, Düngen HD. Systemic inflammation and functional capacity in elderly heart failure patients. Clin Res Cardiol. 2018 Apr;107(4):362-367. doi: 10.1007/s00392-017-1195-x. Epub 2018 Feb 2.

Contribution: Study design, data collection, ensuring the data quality, data analysis, interpretation of data, preparation of all the figures and tables, writing the manuscript

Signature, date and stamp of the supervising University teacher	Signature of the doctoral candidate
_____	_____

Publication 1:

Edelmann F, Musial-Bright L, Gelbrich G, Trippel T, **Radenovic S**, Wachter R, Inkrot S, Loncar G, Tahirovic E, Celic V, Veskovic J, Zdravkovic M, Lainscak M, Apostolović S, Neskovic AN, Pieske B, Düngen HD. Tolerability and Feasibility of Beta-Blocker Titration in HFpEF Versus HFrEF: Insights From the CIBIS-ELD Trial. JACC Heart Fail. 2016 Feb;4(2):140-9.

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Publication 2:

Düngen HD, Tscholl V, Obradovic D, **Radenovic S**, Matic D, Musial-Bright L, Tahirovic E, Marx A, Inkrot S, Hashemi D, Veskovic J, Apostolovic S, von Heahling S, Doehner W, Cvetinovic N, Lainscak M, Pieske B, Edelmann F, Loncar G, Trippel T. Prognostic performance of serial in-hospital measurements of copeptin and multiple novel biomarkers among patients with worsening heart failure: Results from the MOLITOR study. ESC Heart Fail. 2018 Apr;5(2):288-296. doi: 10.1002/ehf2.12231. Epub 2018 Feb 24

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Publication 3:

Radenovic S, Loncar G, Apostolovic S, Zdravkovic M, Veskovic J, Karlicic V, Tahirovic E, Butler J, Düngen HD. Systemic inflammation and functional capacity in elderly heart failure patients. Clin Res Cardiol. 2018 Apr;107(4):362-367. doi: 10.1007/s00392-017-1195-x. Epub 2018 Feb 2.

Impact factor 2016: 4.760

DOI link: https://doi.org/10.1007/s00392-017-1195-x

10. Curriculum Vitae

My Curriculum Vitae does not appear in the electronic version of my thesis due to data protection laws.

11. Publication List

1. **Radenovic S**, Loncar G, Apostolovic S, Zdravkovic M, Veskovic J, Karlicic V, Tahirovic E, Butler J, Düngen HD. Systemic inflammation and functional capacity in elderly heart failure patients. Clin Res Cardiol. 2018 Apr;107(4):362-367. doi: 10.1007/s00392-017-1195-x. Epub 2018 Feb 2.
2. Düngen HD, Tscholl V, Obradovic D, **Radenovic S**, Matic D, Musial-Bright L, Tahirovic E, Marx A, Inkrot S, Hashemi D, Veskovic J, Apostolovic S, von Heahling S, Doehner W, Cvetinovic N, Lainscak M, Pieske B, Edelmann F, Loncar G, Trippel T. Prognostic performance of serial in-hospital measurements of copeptin and multiple novel biomarkers among patients with worsening heart failure: Results from the MOLITOR study. ESC Heart Fail. 2018 Feb 24. doi: 10.1002/ehf2.12231
3. Zelenak C, **Radenovic S**, Musial-Bright L, Tahirovic E, Sacirovic M, Lee CB, Jahandar-Lashki D1, Inkrot S, Trippel TD, Busjahn A, Hashemi D, Wachter R, Pankuweit S, Störk S, Pieske B, Edelmann F, Düngen HD. Heart failure awareness survey in Germany: general knowledge on heart failure remains poor. ESC Heart Fail. 2017 Aug;4(3):224-231.
4. Weisrock F, Fritschka M, Beckmann S, Litmeier S, Wagner J, Tahirovic E, **Radenovic S**, Zelenak C, Hashemi D, Busjahn A, Krahn T, Pieske B, Dinh W, Düngen HD. Reliability of peripheral arterial tonometry in patients with heart failure, diabetic nephropathy and arterial hypertension. Vasc Med. 2017 Aug;22(4):292-300.
5. Edelmann F, Musial-Bright L, Gelbrich G, Trippel T, **Radenovic S**, Wachter R, Inkrot S, Loncar G, Tahirovic E, Celic V, Veskovic J, Zdravkovic M, Lainscak M, Apostolović S, Neskovic AN, Pieske B, Düngen HD. Tolerability and Feasibility of Beta-Blocker Titration in HFpEF Versus HFrEF: Insights From the CIBIS-ELD Trial. JACC Heart Fail. 2016 Feb;4(2):140-9.

12. Affidavit

I, Sara Radenovic, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “Biomarkers in chronic and worsening heart failure patients: Results from the CIBIS-ELD trial and the MOLITOR trial”. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

_____

Date Signature

13. Acknowledgments

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