

Predicting clinical outcomes with novel biomarkers in heart failure patients
after cardiac resynchronization therapy

PhD thesis

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Budapest
2020

1. Introduction

The pathophysiology of chronic heart failure (HF) is compound and the worsening cardiac function leads to multiple organ dysfunctions, including renal dysfunction. The consequent renal failure further worsens the survival of chronic HF patients, including patients, who receive cardiac resynchronization therapy (CRT). Nevertheless, the benefit of CRT has been acknowledged, CRT decreases the volume overload and consequently improves the renal function. Hyperuricemia - the result of the decreased renal function - has been considered as an important sign that projects a poor outcome among heart failure patients on conservative medical therapy.

Vitamin D, initially known as a key regulator hormone of bone metabolism, has several extraskeletal physiologic functions. Based upon recent studies Vitamin D is an important modulator of the renin-angiotensin-aldosterone system (RAAS), inflammatory cytokines and extracellular matrix (ECM) turnover. Moreover, Vitamin D deficiency directly contributes to pathogenesis and worsening of HF by the loss of above modulating mechanisms, causing remodelling of the heart. Numerous cross-sectional and longitudinal studies showed that Vitamin D deficiency is significantly associated with increased risk of HF, in addition worse prognosis of already diagnosed HF was also demonstrated.

The tumour marker carbohydrate antigen-125 (CA-125) is used for screening, diagnosis and monitoring the treatment of ovarian cancer, and elevated levels were found in various types of malignancies, ascites, pleural effusion and other types of peripheral oedema. Furthermore, it reflects the haemodynamic status of the heart and correlates with fluid overload and clinical outcomes in HF patients.

Pentraxin-3 (PTX-3) acts as a mediator of innate immunity and is produced mostly at the site of inflammation. PTX-3 is an important element of humoral innate immunity and was associated with clinical outcomes and disease severity in HF patients with reduced and preserved left ventricular ejection fraction (LVEF).

Fractalkine has been regarded as a complex inflammatory mediator, functioning as an adhesion molecule in its membrane-bound form and as a chemoattractant in its soluble form. Elevated levels were found in HF, and this is independently associated with increased mortality.

Hepatocyte growth factor (HGF), a classic growth factor produced by a wide range of cell types, exerts angiogenetic, antifibrotic and antiapoptotic effects; therefore, it could be considered a multipotent cardioprotective agent. This protective role was justified in a wide range of animal models using various methods of gene transfer. Elevated levels of HGF were found in acute and chronic HF patients, and surprisingly, this predicted worse clinical outcomes. Taking these findings into consideration, elevated levels of HGF may reflect the failing of the protective pathways of the cardiovascular system, therefore identifying the patients in the most unstable clinical condition and at the highest risk.

Matrix metalloproteinase-9 (MMP-9) has a leading role in myocardial remodelling by degrading extracellular matrix proteins. Along with other factors, it was found to have predictive value in ischaemic and dilated cardiomyopathy.

CRT is an effective treatment for patients with HF, severely reduced ejection fraction and wide QRS complex. CRT increases ejection fraction and reduces left ventricular volumes, resulting in reverse remodelling of the heart. Functional capacity of the patients improves, while the morbidity and mortality decrease. Despite optimal patient selection, approximately 20-40% of the patients do not respond to CRT. The demand for novel biomarkers that can identify such patients is therefore crucial.

2. Objectives

The detailed purpose of our research were the following:

1. to assess the ability of pre-implant serum uric acid levels to predict the outcome following CRT. We also intended to test, if the predictive value of uric acid was independent of N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) and other relevant clinical factors.
2. to determine the predictive value of Vitamin D deficiency on the long term mortality after CRT and confirm the association with poor mid-term clinical response.
3. to assess the ability of novel heart failure biomarkers in the prediction of clinical outcomes after CRT. We hypothesized that baseline levels of CA-125, PTX-3, fractalkine, HGF and MMP-9 would predict the six-month reverse remodelling and five-year all-cause mortality in HF

patients undergoing CRT. We also hypothesized that the prediction could be independent of (and therefore offer additional value to) NT-pro-BNP, which is regarded as the gold standard blood biomarker in HF. We aimed to prove our hypotheses with reclassification models.

3. Methods

141 consecutive chronic HF patients were enrolled to our prospective, single-centre observational study. All patients provided their written informed consent before enrolment. The investigation conformed with the principles outlined in the Declaration of Helsinki. The study protocol was approved by the local Ethics Committee.

The patients underwent CRT implantation between September 2009 and December 2010 according to current clinical guidelines in the Heart and Vascular Centre of Semmelweis University, Budapest. Inclusion criteria included stable chronic HF, optimal medical therapy, New York Heart Association functional class II-IVa, wide QRS (>120 msec) in the electrocardiogram (ECG) irrespective of morphology and severely reduced left ventricular ejection fraction (under 35%). We regarded severe systemic inflammatory and hematologic diseases and active malignancies as exclusion criteria.

The follow-up period lasted five years, with visits at six months, two years and five years after CRT implantation. Clinical examinations, laboratory measurements, ECG and echocardiography at baseline were performed. At each follow-up visit, we evaluated the patients' functional status by assessing the NYHA classification, their medical therapy and relevant adverse medical events. Repeated laboratory measurements, echocardiography and ECG were taken at six months.

The primary endpoint of the study was the five-year all-cause mortality in all of our sub-studies. We considered the six-month reverse remodelling, defined as at least a 15% decrease in left ventricular end-systolic volume, as a secondary end-point in the Novel Biomarkers sub-study. Good clinical response, defined as an at least 15% increase of LVEF after six months of CRT, was considered as the secondary endpoint in the Hyperuricemia and Vitamin D deficiency sub-study.

We enrolled 141 patients, according to the exclusion criteria 5 patients were excluded, therefore we recorded the data of 137 patients. The

echocardiographic data of 1 patient was not completed in the Novel Biomarkers and Vitamin D deficiency sub-studies, hence 136 patients were included in the analysis. The routine laboratory parameters were completely collected in 129 patients, their data were analysed.

Exposure to sunlight was determined by the cumulative hours of sunshine in the 30 days preceding enrolment, based upon the public databases of the National Meteorological Service.

Echocardiographic measurements were carried out by licensed echocardiographic experts using a Phillips iE 33 system. LVEF was calculated using Simpson's biplane method, and the left ventricular end-diastolic and end-systolic volumes were calculated using the Teicholz method. To demonstrate the reproducibility of echocardiographic measurements we defined the interobserver and intraobserver variability with Lin's concordance correlation coefficient using 12-12 pair of sample data, which proved substantial correlation [interobserver variability: $\rho_c=0.956$ (0.89-0.98); intraobserver variability: $\rho_c=0.96$ (0.89-0.97)].

We obtained venous blood samples from the patients, then processed the serum and ethylenediaminetetraacetic acid plasma aliquots within two hours. Samples were stored at $-80\text{ }^{\circ}\text{C}$ for later biomarker measurements. Laboratory parameters of renal function were assessed by absorbance photometric techniques on a chemical analyzer [Cobas Integra 400 Plus® (Roche Diagnostics, Mannheim, Germany)]. The following Roche Diagnostics reagents were used: creatinine (Cat. no.: 04810716190); uric acid (Cat. no.: 03183807190); BUN (blood urea nitrogen, Cat. no.: 04460715190), calcium (Cat. no.: 05061482190). NT-proBNP (Roche Elecsys NT-proBNP II kit, Cat. No.: 04842464190, Roche Diagnostics) and total serum 25(OH)-Vitamin D levels (Roche Elecsys Vitamin D total Kit, (Cat. no.: 05894913190, Roche Diagnostics) were measured with immune chemiluminescence technology using a Cobas e 411 (Roche Diagnostics) immunochemical analyser. Commercially available enzyme-linked immunosorbent assays (ELISA) were used for measuring fractalkine (Human CX3CL1/Fractalkine Quantikine ELISA Kit, R&D Systems, Minneapolis, USA, Cat. No.: RD-DCX310), PTX-3 (Human Pentraxin3 / TSG-14 ELISA System, CosmoBio, Tokyo, Japan, Cat. No.: PPX-PP-PD03-E0), HGF (Human HGF Quantikine ELISA Kit, R&D Systems, Cat. No.: RD-DHG00) and MMP-9 (Human MMP-9, Quantikine ELISA Kit, R&D Systems, Cat.

No.: RD-DMP900) plasma levels. Carbohydrate antigen-125 was measured by Liaison CA 125 II immune chemiluminescence assay (Cat. No.: 314211) on a Liaison Analyzer (DiaSorin, Saluggia, Italy).

The data were expressed as the median with interquartile ranges or as percentages with event numbers. Continuous variables were compared using the Wilcoxon matched pair test and the Mann-Whitney test, as appropriate. The Chi squared test was applied for categorical data comparisons. Univariate logistic and Cox regression analyses were used to determine the baseline predictors of the clinical response and the 5-year mortality. The continuous variables were standardized by one standard deviation (SD) increase. Receiver operating characteristic (ROC) analysis was used, and we dichotomized the continuous variables and then compared the Kaplan-Meier curves by using log-rank tests. In the multivariable regression models, the baseline model included variables with $p < 0.05$ value from the univariate analysis, and further adjusted models were built in a forward stepwise manner. Finally, we performed reclassification analyses including C-statistics, net reclassification improvement (NRI) and integrated discrimination improvement (IDI).

A two-tailed p -value of < 0.05 was considered statistically significant. The statistical analysis was carried out using IBM SPSS 22 (Apache Software Foundation, USA), GraphPad Prism 6.03 (GraphPad Software, Inc., USA) and PASS 2008 (NCSS, USA) the open source R software (R version 3.1.2 with PredictABEL and pROC packages).

4. Results

4.1. Hyperuricemia predicts adverse clinical outcomes after cardiac resynchronization therapy

4.1.1. Baseline characteristics

The baseline serum levels of the laboratory parameters are listed below: creatinine 79 (70-91) $\mu\text{mol/L}$, uric acid 432 (331-516) $\mu\text{mol/L}$, BUN 5.9 (4.5-6.5) mmol/L , NT-proBNp 2608 (1331-5101) pmol/L .

4.1.2. Prediction of the 5-year mortality

During a median follow-up time of 1796 (922-2023) days, a total of 55 patients (43%) died. Survival was significantly better in patients with

LBBB morphology and on beta-blocker therapy before CRT implantation. The need for loop diuretics worsened the survival.

The univariate Cox regression analysis showed that the 5-year mortality was predicted by the elevated baseline concentrations of NT-proBNP, creatinine, BUN and uric acid. We set up a basic multivariable model that included NT-proBNP and the relevant clinical variables (LBBB, beta-blocker and loop diuretic therapy, NYHA III-IV class, age and male gender), and then made adjustments to creatinine, BUN and uric acid separately. Due to high internal correlation with baseline serum uric acid levels, baseline creatinine ($r=0.35$; $p<0.001$) and BUN ($r=0.28$; $p=0.0016$) were not included to the multivariate model. A serum uric acid concentration > 386 $\mu\text{mol/L}$ before CRT increased the 5-year mortality risk almost up to 2.4-fold [HR = 2.39 (1.30-4.39), $p < 0.01$] with a negative predictive value of 73%. Moreover, BUN, but not creatinine predicted mortality after the multivariate adjustments [HR 1.39 (1.12-1.75), $p < 0.01$].

4.1.3. Prediction of the 6-month clinical response

On the basis of the criterion, a total of 85 patients (70%) experienced favourable clinical response. Analysis of the clinical parameters showed that decreased baseline ejection fraction resulted in higher risk for poor clinical response. The use of mineralocorticoid receptor inhibitor therapy was associated with a good clinical response as well. Male gender, hypertension and left ventricular volumes also showed a strong tendency in the prediction of poor response ($p < 0.1$).

Univariate logistic regression analysis reported that elevated baseline levels of NT-proBNP, creatinine, BUN and uric acid predicted poor clinical response. We set up a basic multivariable model that included NT-proBNP and all the relevant clinical variables (LVEF, mineralocorticoid receptor inhibitor therapy, male gender, and hypertension). Due to the high internal correlation with baseline ejection fraction, the baseline left ventricular end-systolic ($r = -0.52$; $p < 0.001$) and end-diastolic volumes ($r = -0.44$; $p < 0.001$) were excluded from the multivariable model. After separate adjustments to creatinine, BUN and uric acid, all parameters remained significant predictors. Due to high internal correlation with baseline serum uric acid levels, baseline creatinine ($r=0.35$; $p<0.001$) and BUN ($r=0.28$; $p=0.0016$) were not included to the multivariate model. A serum uric acid

concentration > 386 $\mu\text{mol/L}$ increased the risk of non-response almost up to 3-fold [OR = 2.89 (1.22-6.87), $p = 0.01$], with a negative predictive value of 82%.

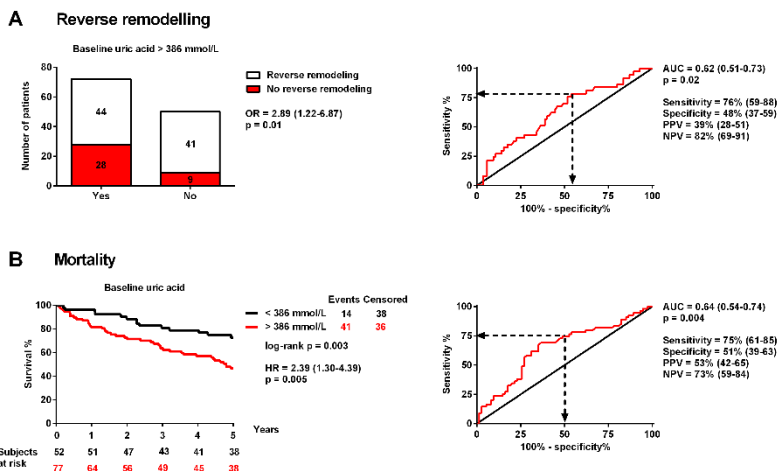


Figure 1. Prediction of the 6-month clinical response and the 5-year mortality. The optimal cut-off value was determined by using receiver operating characteristic (ROC) analysis (right side).

A: Clinical response, visualized by the contingency bar plot, was defined as a relative increase of at least 15% in the LVEF after 6 months of CRT without death (left side). Poor clinical response was tested by using logistic regression analysis.

B: Kaplan-Meier survival curves were analysed with log-rank tests (left side). The 5-year mortality was tested by using Cox regression analysis. The odds and hazard ratios refer to the presence versus the absence of a baseline serum uric acid level > 386 $\mu\text{mol/L}$. OR = odds ratio; HR = hazard ratio; AUC = area under the curve; PPV = positive predictive value; NPV = negative predictive value.

4.2. Vitamin D deficiency predicts poor clinical outcomes in heart failure patients undergoing cardiac resynchronization therapy

4.2.1. Baseline characteristics, effects of CRT on the study population

At baseline deceased patients had higher LVEF and NT-proBNP, while the proportion of patients with LBBB and beta-blocker therapy was significantly lower. Baseline Vitamin D level of the patients were 20.9 ng/mL (15.2-31.7) and we observed no significant change after six months of CRT [6 months: 21.5 (16.2-28.3), $p=0.43$]. LVEF, left ventricular end-systolic and end-diastolic volumes (LVESV and LVEDV) decreased significantly.

4.2.2. Association of baseline Vitamin D concentrations with clinical outcomes

58 patients (43%) deceased during the 5-year follow-up, those who survived had significantly higher baseline Vitamin D levels at baseline [23.07 (16.58-31.73) vs. 18.3 (13.81-23.75) ng/mL, $p=0.018$]. We observed the lack of good clinical response six months after CRT implantation in 45 patients (33%), similarly we measured increased baseline Vitamin D levels in patients with good clinical response [22.56 (15.6-31.87) vs. 18.12 (13.95-23.43) ng/mL, $p=0.027$].

To establish an optimal cut-point for the further assessment of the clinical outcomes, we used ROC analysis. Plasma Vitamin D below 24.13 ng/mL seemed to be an optimal cut-point for 5-year mortality [AUC=0.62 (0.52-0.71), $p=0.018$; sensitivity: 78% (65-87); specificity: 45% (34-58)] and lack of 6-month clinical response [AUC=0.62 (0.52-0.71), $p=0.027$; sensitivity: 80% (65-90); specificity: 42% (32-53)] as well.

After that, we created groups of patients with baseline Vitamin D below and over 24.13 ng/mL. The use of beta-blockers was the only parameter, that differed significantly between the sub-groups (98% vs. 85%, $p=0.015$). Sunlight exposure before enrolment did not differ significantly between the groups ($p=0.56$). When analysing the severity of heart failure, there was no difference between groups at baseline reflected by the NT-proBNP levels [2626 (1683-5214) vs. 2518 (988-4791), $p=0.18$] or the NYHA class ($p=0.61$). NT-proBNP levels remained significantly elevated only in the subgroup of patients with low Vitamin D levels [1216 (337-2214) vs. 2116 (927-3865) $p=0.019$]. Moreover, their functional status (NYHA class) also did not improve ($p=0.001$).

4.2.3. Predictors of 5-year mortality and lack of good clinical response

We analysed the 5-year all-cause mortality using univariate Cox regression and the six-month clinical response by univariate logistic regression analysis. Vitamin D level lower than 24.13 ng/mL was significantly associated with increased risk of long-term mortality [HR: 2.25 (1.21-4.17), $p=0.008$] and lack of good clinical response [OR: 2.51 (1.11-5.68), $p=0.027$].

LBBB ($p<0.0001$), use of beta blocker therapy ($p=0.003$) and increasing NT-proBNP levels ($p<0.0001$) predicted all-cause mortality,

increasing age showed a tendency ($p=0.07$). Relevant baseline clinical variables related to the lack of good clinical response were hypertension ($p=0.08$), hyperlipidaemia ($p=0.09$), mineralocorticoid receptor inhibitor therapy ($p=0.06$) and increasing levels of NT-proBNP ($p=0.22$).

To demonstrate the independent influence of decreased Vitamin D levels on mortality, we set up a basic multivariable Cox regression model with the baseline clinical variables shown to be relevant by the univariate analysis ($p<0.10$). The baseline multivariable model included age, LBBB, use of beta blocker therapy and baseline NT-proBNP. In the next step, we entered Vitamin D levels into the baseline model in a forward stepwise way. Vitamin D levels under 24.13 ng/mL predicted mortality, in the multivariable model as well [HR=1.92 (1.02-1.45), $p=0.045$].

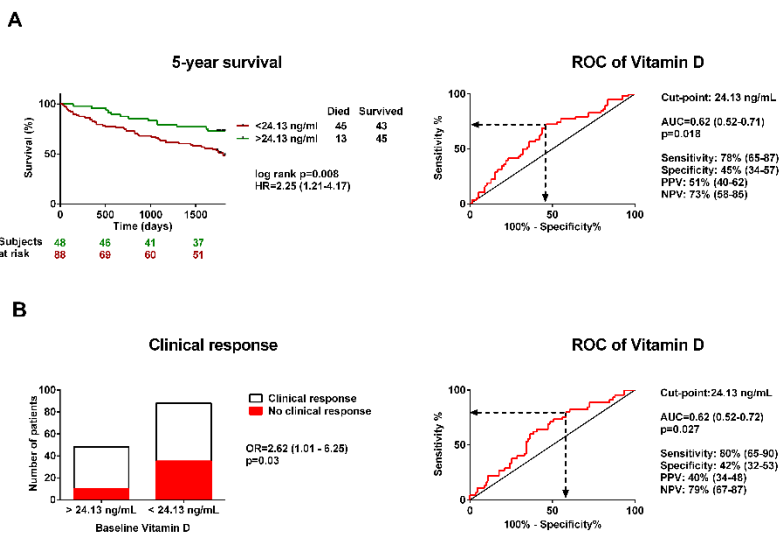


Figure 2. Impact of baseline Vitamin D on five-year mortality and clinical response.

ROC analysis was performed for determining the optimal cut-off point for baseline plasma Vitamin D levels. The odds and hazard ratios refer to the presence versus the absence of a baseline Vitamin D level < 24.13 ng/mL.

A: We compared the Kaplan-Meier survival curves by log rank test in groups of baseline plasma Vitamin D levels below and over 24.13 ng/mL. We tested the 5-year mortality using Cox regression analysis.

B: Clinical response, defined as a relative increase of at least 15% in the LVEF 6 months after CRT implantation, was visualized by the contingency bar plot. We tested the lack of clinical response using logistic regression analysis.

The same method was used to investigate the clinical response. We included all the relevant factors to the basic multivariable model: hypertension, hyperlipidaemia, mineralocorticoid receptor inhibitor therapy and increasing levels of NT-proBNP. We entered Vitamin D levels into the basic model in a forward stepwise way. Similarly to mortality prediction, Vitamin D was an independent predictor of lack of good clinical response [OR=2.62 (1.01-6.25), p=0.03].

4.3. Novel biomarkers in cardiac resynchronization therapy: Hepatocyte growth factor is an independent predictor of clinical outcome

4.3.1. Study population, effects of CRT on echocardiography results and biomarkers

The median age of the patients was 67 (60-73) years, and 80% were male. The median QRS duration was 163 (141-184) msec, and 82% of the patients had LBBB morphology in the ECG. After six months of CRT, the LVEF increased significantly [28% (23-33) vs 37% (31-41), p<0.0001], while the left ventricular end-diastolic volume and left ventricular end-systolic volume decreased [left ventricular end-diastolic volume: 303 (251-351) mL vs 259 (203-332) mL, p<0.0001; left ventricular end-systolic volume: 303 (154-276) vs 167 (116-242) mL, p<0.0001].

We investigated the change of the studied biomarkers after six months of CRT. Statistically significant lower plasma levels of pentraxin-3 [4.92 (3.29-6.84) ng/mL vs 3.13 (2.39-4.48) ng/mL, p<0.0001], NT-proBNP [2612 (1377- 5124) pg/mL vs 1626 (725-3300) pg/mL, p<0.001], HGF [1379 (1029-1863) pg/mL vs 1083 (862-1328) pg/mL, p<0.001] and carbohydrate antigen-125 [22.75 (11.05-69.90) U/L vs 18.30 (9.15-34.35), p<0.001] were measured after six months of follow-up. Plasma levels of fractalkine and matrix metalloproteinase-9 remained unaltered.

4.3.2. Association of biomarker concentrations with clinical response

A total of 70 patients (51%) were considered as responders, and 66 patients (49%) were non-responders according to the criteria (>15% relative decrease in left ventricular end-systolic volume). NT-proBNP levels were significantly higher in non-responder patients at baseline (p=0.0002) and at six months (p<0.0001). Moreover, we observed a significant decrease of plasma NT-proBNP levels only in case of responders (p=0.0001). PTX-3 and

CA-125 levels were considerably higher in non-responders at baseline (PTX-3: $p=0.0005$; CA-125: $p=0.001$) and six months (PTX-3: $p=0.02$; CA-125: $p=0.002$). After six months of CRT, plasma levels decreased both in responders (PTX-3: $p=0.0001$; CA-125: $p=0.01$) and non-responders (PTX-3: $p<0.0001$; CA-125: $p=0.02$). Patients with a lack of reverse remodelling after CRT had significantly higher baseline HGF ($p=0.001$) and fractalkine ($p=0.004$) levels. HGF levels were significantly reduced following CRT (responders: $p=0.003$; non-responders: $p=0.01$), while plasma fractalkine levels did not change over time. We did not find significant differences in MP-9 levels between the groups, although we observed a significant rise in responders after six months ($p=0.003$).

4.3.3. Univariate predictors of mortality and reverse remodelling

We examined the 5-year all-cause mortality by univariate Cox regression and the six-month reverse remodelling using univariate logistic regression analysis. During the five years of follow-up, 58 patients (43%) deceased. All-cause mortality was significantly related to absence of LBBB ($p<0.0001$), or beta blocker therapy ($p=0.003$) and increasing NT-proBNP levels ($p<0.0001$), while increasing age showed a tendency ($p=0.07$). Out of the novel biomarkers, increasing levels of fractalkine ($p=0.01$), HGF ($p<0.0001$) and MMP-9 ($p=0.04$) predicted the five-year mortality.

A total of 70 patients (51%) experienced reverse remodelling at 6 months based on the echocardiographic criterion. Increasing age ($p=0.004$), New York Heart Association class III-IV ($p=0.01$) and increasing levels of NT-proBNP ($p=0.004$) predicted the lack of six-month reverse remodelling. A tendency was seen between ischaemic aetiology of HF ($p=0.069$), mineralocorticoid receptor inhibitor therapy ($p=0.08$) and a lack of response. Increasing plasma levels of fractalkine ($p=0.04$), PTX-3 ($p=0.01$) and HGF ($p=0.01$) predicted the lack of response to CRT.

4.3.4. Multivariable risk prediction models of mortality and reverse remodelling

To further investigate the impact of novel biomarkers on mortality, we set up a basic multivariable Cox regression model with all the variables previously shown to be relevant by the univariate analysis. The baseline multivariable model included age, LBBB, beta blocker therapy and NT-

proBNP. We entered the novel biomarkers separately into the baseline model in a forward stepwise way in the next step. Only HGF predicted mortality in the multivariable model as well [HR=1.35 (1.11-1.64), $p=0.003$, per 1 SD increase].

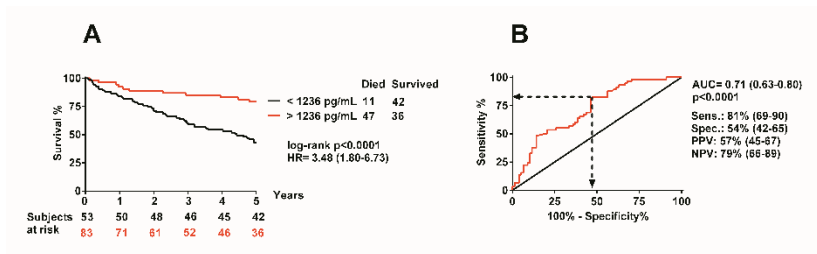


Figure 3. Impact of baseline HGF levels on the five-year mortality.

A: Comparison of the Kaplan-Meier survival curves using log rank test. The survival of patients with baseline plasma HGF levels below and over 1236 pg/mL was analysed.

B: ROC analysis was performed to determine the optimal cut-off point for baseline plasma HGF levels.

We used the same approach to investigate the reverse remodelling. We included all relevant factors to the basic multivariable model: age, ischaemic aetiology of HF, NYHA class III-IV, mineralocorticoid receptor inhibitor therapy and NT-proBNP. Next, we entered the novel biomarkers separately into the baseline model in a forward stepwise way. Similarly, to the mortality prediction, HGF was the only independent predictor of reverse remodelling [OR=1.83 (1.10-3.04), $p=0.01$, per 1 SD increase].

4.3.5 Assessment of the additional benefit of HGF in the prediction of outcome

We used ROC analysis to establish an optimal cut-point for the further assessment of the outcomes. We aimed to demonstrate a clinically relevant cut-off point with the possibly highest sensitivity associated with the highest possible sensitivity. Plasma HGF levels exceeding 1236 pg/mL seemed to be an optimal cut-point [AUC=0.71 (0.63-0.80), $p < 0.0001$; sensitivity: 81% (69-90); specificity: 54% (45-65)]. HGF levels more than 1236 pg/mL were associated with an increased risk of long term mortality [HR=3.48 (1.80-6.73), $p < 0.0001$].

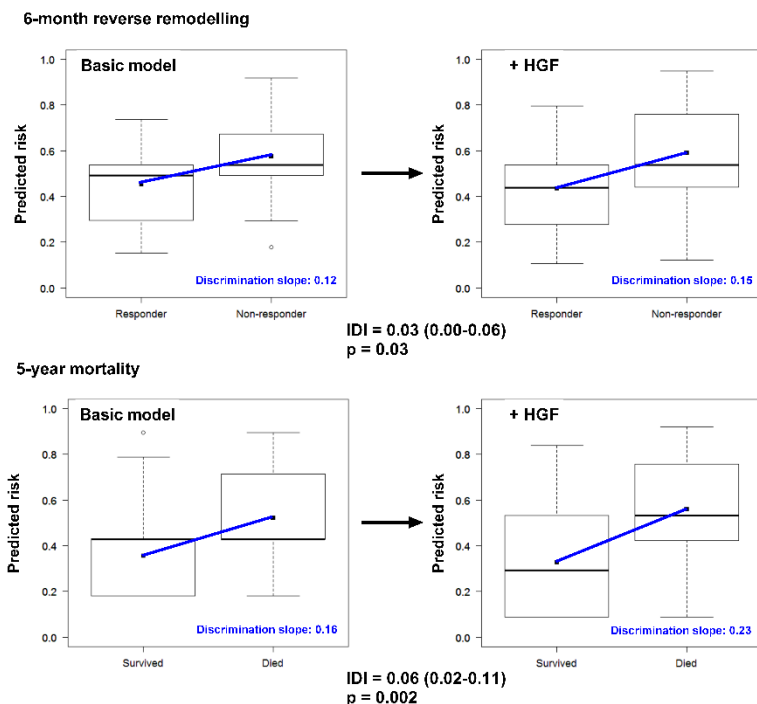


Figure 4. Improved discrimination capacity of the prediction models. The improved discrimination capacity is visualized by a box and whiskers diagram. The discrimination slope is described as the difference in the mean probabilities of events minus non-events (upper panel: 6-month reverse remodelling; lower panel: 5-year mortality). Adding the HGF to the basic model increases the discrimination slope (blue line) of the prediction models. The difference in the discrimination slopes is the IDI (integrated discrimination improvement). HGF = hepatocyte growth factor.

When HGF was added to the baseline multivariable model of mortality [NRI=0.69 (0.39-0.99), $p < 0.0001$; IDI=0.06 (0.02-0.11)] and the reverse remodelling [NRI=0.39 (0.07-0.71), $p = 0.01$; IDI=0.03 (0.00-0.06)], we observed a significant improvement in reclassification and discrimination. Adding the HGF biomarker to the baseline model increased the discrimination slope of both prediction models (from 0.12 to 0.15 for reverse remodelling; from 0.16 to 0.23 for mortality). The predicted and observed risks, determined by Hosmer-Lemeshow test, did not differ significantly throughout the analyses, which indicated good calibration and confirmed the

validity of the results. The decrease in Brier Score and the simultaneous increase in Nagelkerke's R^2 suggested an improved predictive capacity.

5. Conclusions

The strength of our research lies in the clinical relevance of the studied biomarkers. We investigated novel biomarkers representing various pathways of the complex pathophysiology of HF, with proven predictive capacity in patients with medical therapy. Uric acid and Vitamin D are used widespread in routine medical practice and diagnostics, their prognostic role in HF was also recently demonstrated. Our studies were the first to show the significant association of the aforementioned biomarkers with clinical outcomes after CRT. Synopsis of our key findings:

1. We confirmed the predictive value of serum creatinine and BUN, and we found that the baseline uric acid levels also predict the clinical response and the long-term mortality in CRT. A serum uric acid level > 386 $\mu\text{mol/L}$ before CRT decreases the chance of 6-month good clinical response up to 3-fold and increases the 5-year mortality risk up to 2.4-fold.
2. Vitamin D levels under 24.13 ng/mL predicted long term mortality and poor clinical response in HF patients undergoing CRT, independently of all significant baseline predictors, including NT-proBNP. Moreover, patients with Vitamin D insufficiency had significantly higher NT-proBNP levels and suffered from more severe HF after six months of CRT.
3. Increased HGF levels predict the lack of reverse remodelling and the long-term mortality of HF patients undergoing CRT independently of NT-proBNP and other relevant factors. Reclassification analyses proved that adding HGF to the baseline model resulted in improved prediction performance, discrimination and reclassification in the prediction models. Thus, HGF may be helpful in identifying patients with the highest risk of mortality or insufficient clinical response.

6. Bibliography of the candidate's publications

Sum of impact factors of publications related to the dissertation: 9.125.

Perge P, Boros AM, Zima E, Geller L, Merkely B, Szeplaki G. (2018) Hyperuricemia predicts adverse clinical outcomes after cardiac resynchronization therapy. *Scand Cardiovasc J*, 52: 250-255.

Impact factor: 1.286.

Perge P, Boros AM, Gellér L, Osztheimer I, Szilágyi S, Tahin T, Apor A, Nagy KV, Zima E, Molnár L, Merkely B, Széplaki G. (2019) Vitamin D Deficiency Predicts Poor Clinical Outcomes in Heart Failure Patients Undergoing Cardiac Resynchronization Therapy. *Dis Markers*, 2019: 4145821.

Impact factor: 2.761.

Perge P, Boros AM, Szilagyi S, Zima E, Molnar L, Geller L, Prohaszka Z, Merkely B, Szeplaki G. (2018) Novel Biomarkers in Cardiac Resynchronization Therapy: Hepatocyte Growth Factor Is an Independent Predictor of Clinical Outcome. *Rev Esp Cardiol (Engl Ed)*, 72 (1): 48-55.

Impact factor: 5.078.

Sum of impact factors of publications not related to the dissertation: 37.971.

Széplaki G, Perge P, Merkely B. (2014) A terápiarezisztencia áttörése hipertóniában: a szimpatikus tónus csökkentésének eszközös lehetőségei. *Háziorvosi Továbbképző Szemle*, 19: 399-402.

Boros AM, Szeplaki G, Perge P, Jenei Z, Bagyura Z, Zima E, Molnar L, Apor A, Becker D, Geller L, Prohaszka Z, Merkely B. (2016) The ratio of the neutrophil leucocytes to the lymphocytes predicts the outcome after cardiac resynchronization therapy. *Europace*, 18: 747-754.

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Boros AM, Perge P, Jenei Z, Karady J, Zima E, Molnar L, Becker D, Geller L, Prohaszka Z, Merkely B, Szeplaki G. (2016) Measurement of the Red Blood Cell Distribution Width Improves the Risk Prediction in Cardiac Resynchronization Therapy. *Dis Markers*, 2016: 7304538.

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