Role of laboratory biomarkers in the cardiac resynchronization therapy of heart failure

PhD thesis

András Mihály Boros, M.D.

Semmelweis University
Doctoral School of Basic and Translational Medicine

Supervisor: Gábor Széplaki, M.D., Ph.D.

Official Reviewers: Attila Kardos, M.D., Ph.D.
Eszter Mária Horváth, M.D., Ph.D.

Head of the Final Examination Committee:
Barna Vásárhelyi, M.D., DSc.

Members of the Final Examination Committee:
Járai Zoltán, M.D., Ph.D.
Levente Kiss, M.D., Ph.D.

Budapest
2018

1. Introduction

Chronic heart failure affects multiple organs other than the heart itself and should be not considered only as a reduction in pump function. It activates the immune system and induces global metabolic disturbances, which can be traced by means of various laboratory tests. The systemic inflammation and oxidative stress result in hyperglycemia, while the ongoing inflammation drives the elevations in C reactive protein (CRP) and total protein concentrations. The lipoproteins [cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL)] buffer the inflammatory cytokines and endotoxins, and the chronic inflammation therefore decreases the cholesterol level. The release of catecholamines and sympathetic activation lead to a volume overload and redistribution. The volume overload enhances N-terminal pro-brain natriuretic peptide (NT-proBNP) production, dilates the ventricles and causes cardiac tissue damage and remodelling, resulting in increased levels of creatine kinase MB isoenzyme (CKMB), CKMB / CK and (lactate dehydrogenase) LDH. Since one of the characteristic features of chronic heart failure is reduced mitochondrial energy production in both the cardiac and the skeletal muscle, a creatine phosphate deficit develops with low creatine kinase (CK) activity and a consequently reduced serum level. On the other hand, the volume redistribution decreases the renal and hepatic blood flows. The consequent hepatobiliar dysfunction is reflected by elevated levels of liver enzymes [glutamic-oxaloacetic transaminase (GOT), glutamat-pyruvat transaminase GPT, gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP)] and total bilirubin, and decreased albumin production, while the renal dysfunction is characterized by increases in the levels of serum creatinine, uric acid and blood urea nitrogen (BUN).

The neurohormonal activation, oxidative stress, and the chronic inflammation in heart failure increase the catecholamine release and the plasma cortisol levels leading to bone marrow suppression and down-regulation of the lymphocyte proliferation and differentiation with aggravated lymphocyte apoptosis. On the other hand, the inflammatory citokines directly promote the neutrophil release to the circulation from the bone marrow. Consequently, the ratio of the neutrophil leucocytes to the lymphocytes (NL-ratio) is elevated in heart failure, which impairs prognosis.

The upregulated plasma corticosteroids suppress the haematopoiesis as well in the bone marrow leading to anemia and increased red blood cell distribution width (RDW). The RDW increase has been also linked to a poor survival in heart failure patients. The complement system connects the innate and the acquired immunity and is responsible for the maintenance of the immunohomeostasis. Recently, it has been proposed that the complement system plays a role in the pathogenesis of chronic heart failure, a study described elevated levels of complement factor 3 (C3), activated complement factor 3 (C3a) and soluble membrane attack complex (sC5b9) in heart failure patients and increasing C3a levels impaired the survival chances.

The cardiac resynchronization therapy (CRT) is highly effective treatment for the medically refractory chronic heart failure patients with intra- and interventricular conduction delay by biventricular pacing. Most of the patients respond adequately to the therapy with improvement of the functional status and the reverse remodelling of the failing heart. Biomarkers are predictive factors that could predict a response to a therapy, for instance to CRT, with statistical probability and therefore are able to make therapeutic decisions. The American Heart Association declared the criteria for evaluations of novel biomarker in 2009: measures of both discrimination and precision should be reported and incremental information when added to standard risk markers should be documented. C-statistics displays discrimination, while precision is characterised by calibration and performance. Incremental value can be shown via novel reclassification and discrimination analyses including net reclassification improvement and integrated discrimination improvement.

2. Objectives

The purpose of our research was to evaluate the role of laboratory parameters in chronic heart failure patients undergoing CRT.

Consequently, we set out to

1. Investigate how serum biomarkers, including cardiovascular (NT-proBNP, CK, CKMB, CKMB / CK, LDH), renal (creatinine, uric acid and BUN), inflammatory (CRP, total protein), hepatobiliary (GOT, GPT, GGT, ALP, total bilirubin, albumin) and metabolic (cholesterol, triglyceride, HDL, LDL, glucose) markers can mirror the multi-organ improvement after CRT. We hypothesized that CRT is associated with systemic biochemical changes 6 months and 2 years after implantation that correlate with echocardiographic improvement.

2. Study the predictive role of NL-ratio in the resynchronization therapy of chronic heart failure. We hypothesized that CRT would decrease the NL-ratio and elevated NL-ratio before CRT would predict the lack of 6-month reverse remodelling and the 2-year mortality of the patients independently of the baseline NT-proBNP levels or other factors.

3. Examine the predictive role of RDW in the resynchronization therapy of chronic heart failure. We hypothesized that CRT would alter the RDW and increased RDW before CRT would predict the lack of 6-month reverse remodelling and the 5-year mortality of the patients independently of the baseline NT-proBNP levels or other factors.

4. Explore the impact of the complement components (C3, C3a, sC5b9) in the resynchronization therapy of chronic heart failure. We hypothesized that CRT would decrease complement activation and increased C3, C3a, and sC5b9 before CRT would predict the lack of 6-month reverse remodelling and the 5-year mortality of the patients independently of the baseline NT-proBNP levels or other factors.

5. Link the results derived from the analyses of NL-ratio, RDW and complement components and investigate their co-effect in the prediction.

3. Methods

A total of 141 consecutive patients with medically refractory heart failure referred to our Heart and Vascular Center between September 2009 and December 2010 for CRT implantation, according to the current guidelines. This work was supported by the Hungarian Foundation Programs "Semmelweis Egyetem Híd Projekt" (TÁMOP-4.2.2-08/1/KMR-2008-0004) and "Semmelweis Egyetem Magiszter Program" (TÁMOP-4.2.2./B10/1.-210-0013), János Bolyai Research Scholarships of the Hungarian Academy of Sciences (to Gábor Széplaki and László Gellér), the Hungarian Scientific Research Fund (OTKA K 105555) and Research Funds of the Doctoral School of Semmelweis University (to András Mihály Boros and Péter Perge). Prior to the enrolment, the local Ethics Committee of the Semmelweis University had approved the protocol, which was in accordance with the Helsinki Declaration, and all of the patients provided their written informed consent.

The inclusion criteria included previously diagnosed and medically treated chronic heart failure [New York Heart Association (NYHA) class II–IV] with wide QRS in electrocardiogram (ECG) (120 ms) and a severely reduced (<35%) left ventricular ejection fraction (LVEF). We considered autoimmune diseases, haematologic diseases, acute or chronic inflammatory diseases, and malignancies as exclusion criteria, and three patients were excluded on this basis. The procedure of the CRT implantation was performed by implantation of a left ventricular lead into the sidebranch of the coronary sinus, a right ventricular lead in a septal position and a right atrial lead where appropriate. Laboratory tests, clinical examinations, and ECG and echocardiographic measurements were carried out before CRT implantation and 6 months and 2 years later. The optimal medical therapy of chronic heart failure was maintained throughout the 5-year long follow-up period. The all-cause 2-year and 5-year mortality was taken as the end-point of the study. Echocardiographic reverse remodelling was considered as secondary endpoint and was defined as an improvement of at least 15% in the left ventricular endsystolic volume (LVESV) 6 months after CRT implantation.

Complete routine clinical chemistry laboratory profile was available in n = 129 patients before CRT. A total of n = 122 patients had complete

qualitative blood count (NL-ratio) + NT-proBNP results, while n = 134 had complete quantitative blood count (RDW) + NT-proBNP values before CRT. The complement component (C3, C3a, sC5b-9) and NT-proBNP measurement was complete before CRT implantation in n = 126 patients.

Therefore, we separated four investigational sub-populations (routine laboratory, NL-ratio, RDW and complement components) and evaluated them one-by-one in the final analyses. The data of 122 age, gender, and body mass index matched healthy control subjects were also analysed who participated in the voluntary 'Budakalász Study' of our clinic.

The laboratory samples were processed in the Central Laboratory of the Heart and Vascular Center. Serum samples for routine laboratory measurements were analyzed with the Cobas Integra 400 Plus® (Roche Diagnostics, Germany) clinical chemistry system. Blood cell counting was performed by using the Symex XS-1000i® (Kobe, Japan) system. The levels of the NT-proBNP were measured with electrochemiluminescence technology by Cobas e 411® analyser (Roche Diagnostics). Total C3 levels were measured by Roche Cobras Integra 800® (Roche Diagnostics). C3a and sC5b-9 concentrations were determined with enzyme-linked immunosorbent assay kits (A015® and A029® Quidel, USA)

The reported statistical analysis was carried out by using IBM SPSS 22® (Apache Software Foundation, USA), Graphpad Prism 6.03® (Graph-Pad Softwares Inc., USA), PASS 2008® (NCSS, USA), and the open source R® software (R version 3.1.2 with PredictABEL® and pROC® packages). The parameters reported in this study differed from the normal distributions (checked by Shapiro-Wilks test), thus we used non-parametric tests, and the data are expressed as median values with interquartile ranges (25–75%) and as percentages with event numbers. A two-tailed P-value of < 0.05 was considered statistically significant in all cases.

Two continuous variables were compared by using t-tests, while two or more continuous variables were compared by using variance analyses. Categorical data were compared by the Fisher's exact test. Spearman correlation was calculated. The area under the curves (AUC) were assessed by the receiver operating characteristics analysis (ROC) to set up the best fitting cut-off values for the later prediction models. We compared the

Kaplan–Meier survival curves using the log-rank test. The prediction of the reverse remodelling and the mortality was tested by the univariate logistic and Cox regression analyses. The continuous variables were standardized by one standard deviation increase. The reference models of the prediction included the statistically significant variables of the univariate logistic and Cox regression analysis. The point of interest biomarker(s) were entered into the reference model in a forward stepwise way.

We validated the models and performed power analyses. Precision was described by calibration and performance. Calibration was checked by using the Hosmer-Lemeshow test (HL test). Performance of the models was characterized via the changes of the Brier score and Nagelkerke's R^2. Discrimination was computed by the c-statistic with DeLong test, while incremental value was shown via reclassification and discrimination analyses including net reclassification improvement (NRI) and integrated discrimination improvement (IDI).

4. Results

The baseline characteristics of the four investigational sub-populations (routine laboratory, NL-ratio, RDW and complement components) did not differ statistically significant. In the total cohort the median age of the patients was 67 years, 81% of them were male, 57% had a heart failure of ischaemic origin, 82% had left bundle branch block (LBBB) in the ECG and the median QRS width was 160 ms. The median LVEF was 27% and the functional status showed that 86% of the patients were in NYHA class III or IV. The median age of the 122 control subjects was 67 years, 82% was male.

4. 1. Routine laboratory sub-population

4.1.1. Echocardiographic changes

The CRT led to the LVEF being increased statistically significant after 6 months [27% (23-33) vs. 37% (31-41), $p < 0.001$], while the LVESV [210 (153-267) vs. 167 (111-234) mL, $p < 0.001$], and left ventricular end-diastolic volume (LVEDV) [303 (242-351) vs. 259 (202-315) mL, $p < 0.001$] were decreased. No further significant improvement was seen at the 2-year follow-up in the LVEF [42% (32-48), $p = 0.05$], LVESV [160 (109-212) mL, $p = 0.57$] and LVEDV [242 (195-305) mL, $p = 0.60$]. However, the improvement still persisted (baseline vs. 2 years, $p < 0.001$).

4.1.2. Changes of cardiovascular biomarkers

As compared with the healthy controls, the chronic heart failure patients presented with elevated NT-proBNP [2608 (1331-5101) vs. 88 (45-159) pg/mL], increased CKMB [17 (12-24) vs. 13 (11-17) U/L] and LDH [389 (331-476) vs. 303 (273-335) U/L] ($p < 0.001$) activity, but decreased CK [76 (52-109) vs. 112 (85-155) U/L] activity ($p < 0.001$). The CKMB/ CK ratio was also elevated [23 (14-40) vs. 12 (9-16)] ($p < 0.001$) in heart failure patients. Two years following CRT implantation the NT-proBNP concentration decreased [968 (331-2312) pg/mL] and the CK activity increased [108 (74-138) U/L] ($p < 0.001$), while the CKMB [18 (15-23) U/L] and LDH [383 (342-443) U/L] activity remained statistically unchanged ($p = 0.99$ and $p = 0.26$, respectively). Similarly, the CKMB/ CK ratio did not change statistically significant 2 years after CRT [21 (11-37), $p = 0.11$]. The

change in NT-proBNP after 6 months correlated well with those in the LVEF ($r = -0.26$, $p = 0.006$), LVESV ($r = 0.37$, $p < 0.001$) and LVEDV ($r = 0.28$, $p = 0.003$). The change in NT-proBNP after 2 years also correlated with those in the LVEF ($r = -0.42$, $p < 0.001$), LVESV ($r = 0.50$, $p < 0.001$) and LVEDV ($r = 0.55$, $p < 0.001$). Additionally, the change in CK after 2 years correlated with those in the LVESV ($r = 0.34$, $p = 0.009$) and LVEDV ($r = 0.37$, $p = 0.004$).

4.1.3. Changes of renal and inflammatory biomarkers

We have found significant elevations in both renal [creatinine: 107 (79-134) vs. 79 (70-91) μmol/L, uric acid: 432 (331-516) vs. 322 (277-377) μmol/L and BUN: 8.3 (6.4-11.5) vs. 5.9 (4.5-6.5) mmol/L) and inflammatory [CRP: 3.7 (1.7-9.0) vs. 1.7 (1.1-2.9) mg/L and total protein: 76 (72-79) vs. 70 (68-73) g/L] biomarkers in the chronic heart failure patients before CRT ($p < 0.001$). The renal improvement at 2 years following CRT was characterized by decreases in creatinine [89 (83-124 μmol/L; $p = 0.03$), uric acid [340 (290-433) μmol/L, $p < 0.001$] and BUN [6.8 (5.0-9.7) mmol/L, $p = 0.008$]. The anti-inflammatory potential of the CRT was suggested by the reductions in CRP [2.9 (1.2-6.3) mg/L, $p = 0.03$] and total protein [71 (68-73) g/L, $p < 0.001$] concentrations. The change in the serum creatine level at 6 months was associated with an LVESV improvement ($r = 0.24$, $p = 0.01$), while the change in uric acid was followed by an LVEF recovery ($r = -0.17$, $p = 0.04$). The change in the BUN level at 2 years correlated with the ventricular reverse remodelling (LVESV $r = 0.23$, $p = 0.06$, and LVEDV $r = 0.29$, $p = 0.02$). The change in CRP at 2 years paralleled the LVEF improvement ($r = -0.22$, $p = 0.04$).

4.1.4. Changes of hepatobiliary biomarkers

The baseline activity of liver enzymes [GOT: 25 (19-33) vs. 20 (16-24) U/L, GPT: 25 (19-34) vs. 18 (14-24) U/L and GGT: 52 (32-90) vs. 22 (16-31) U/L] and total bilirubin concentration [16 (11-23) vs. 7 (5-10) μmol/L] elevated statistically significant ($p < 0.001$), while that of ALP not [72 (61-98) vs. 71 (60-85) U/L, $p = 0.14$], and the level of albumin [45 (43-48) vs. 46 (44-47) g/L, $p=0.04$] was reduced in the chronic heart failure patients as compared to the control subjects. At 2 years after the CRT, the activity of GOT [21 (17-28) U/L], GPT [18 (14-23) U/L] and GGT [34 (21-

73) U/L] were lowered significantly ($p < 0.001$), that of ALP was unchanged [74 (59-98) U/L, $p = 0.99$] and that of albumin was increased [46 (43-48) g/L, $p = 0.006$]. Of the above parameters, the GGT change at 2 years correlated with the change in the LVEF ($r = -0.32$, $p = 0.005$).

4.1.5. Changes of metabolic biomarkers

As regards the metabolic parameters, the patients had low levels of lipoproteins [cholesterol: 4.3 (3.6-5.0) vs. 5.4 (4.9-6.3) mmol/L, triglyceride: 1.4 (1.0-1.9) vs. 2.0 (1.4-3.0) mmol/L, HDL: 1.1 (0.9-1.4) vs. 1.4 (1.1-1.7) mmol/L, and LDL: 2.5 (2.0-3.2) vs. 3.4 (2.8-4.1) mmol/L], but a high glucose [6.2 (5.6-7.2) vs. 5.6 (5.3-6.3) mmol/L] concentration ($p < 0.001$) at baseline. Following CRT implantation, the levels of lipoproteins increased [cholesterol: 4.6 (3.8-5.4) mmol/L, triglyceride: 1.8 (1.2-3.0) mmol/L, HDL: 1.2 (1.0-1.5) mmol/L, and LDL: 2.7 (2.0-3.3) mmol/L] ($p < 0.001$), while that of the glucose decreased over time [5.7 (5.1-6.8) mmol/L, $p = 0.01$]. The triglyceride changes were followed by reductions in the left ventricular volumes at 2 years (LVESV $r = -0.22$, $p = 0.06$ and LVEDV $r = -0.33$, $p = 0.005$).

4.2. NL-ratio subpopulation

4.2.1. Changes in laboratory parameters 6 months after CRT implantation

The NL-ratio was elevated in chronic heart failure patients when compared with the healthy controls [2.93 (2.12-4.05) vs. 2.21(1.64-2.81), $p < 0.0001$]. We have observed statistically significant decreases in the NL-ratio [2.93 (2.12-4.05) vs. 2.82 (2.05-3.05), $p = 0.04$].

4.2.2. Prediction of the 6-month reverse remodelling

From the aspect of the echocardiographic reverse remodelling following the CRT, 63 patients (52%) were categorized as responders and 59 patients (48%) as non-responders. Responders at baseline had lower NL-ratio [2.62 (1.90-3.71) vs. 3.12 (2.35-4.66), $p = 0.01$] and lower NT-proBNP levels [2315 (1004-3989) vs. 3305 (1869-6019) pg/mL, $p = 0.004$, respectively]. Using the ROC analysis, we defined the NL-ratio 2.95 [AUC = 0.63 (0.53-0.73), $p = 0.01$] and the NT-proBNP 1522 pg/mL [AUC = 0.64 (0.55-0.74), $p = 0.004$] as optimal cut-off values for the prediction models. The univariate logistic regression analysis revealed that the reverse remodelling following

the CRT was significantly associated with younger age, female gender, wider QRS, and better NYHA functional status at baseline, thus these variables composed of the later multivariable basic model. We entered the NT-proBNP to the basic model, then the NL-ratio in a forward stepwise way and the baseline NL-ratio exceeding 2.95 independently predicted the lack of the 6-month reverse remodelling [odds ratio (OR) = 0.38 (0.17-0.85), p= 0.01].

4.2.3. Prediction of the 2-year mortality

Up to a median follow-up period of 787 days, 29 (23%) patients died. Those who survived the follow-up period had lower baseline NL-ratio [2.70 (2.05-3.68) vs. 3.90 (2.87-5.65), p = 0.001] and lower NT-proBNP levels [2418 (1116-4050) vs. 4959 (2429-6864) pg/mL, p = 0.002]. The ROC analysis showed that the predefined NL-ratio of 2.95 [AUC = 0.69 (0.58-0.80), p = 0.001] and the NT-proBNP level of 1522 pg/mL [AUC = 0.70 (0.60-0.80), p = 0.0008] were also optimal for the mortality prediction models. The Kaplan–Meier survival curves based on the NL-ratio are shown in Figure 1. The 2-year survival of the patients in the univariate Cox regression analysis was significantly predicted by the LBBB pattern of the ECG, the use of beta-blocker therapy, the absence of diabetes mellitus, and the non-ischaemic aetiology of the heart failure, and consequently these parameters comprising the basic model of the multivariable analysis. Using the multivariable Cox regression analysis, we have adjusted the NT-proBNP to the basic model, then the NL-ratio in a forward stepwise way and the NL-ratio exceeding 2.95 independently predicted the 2-year mortality of the patients [hazard ratio (HR) = 2.44 (1.04-5.71), p = 0.03].

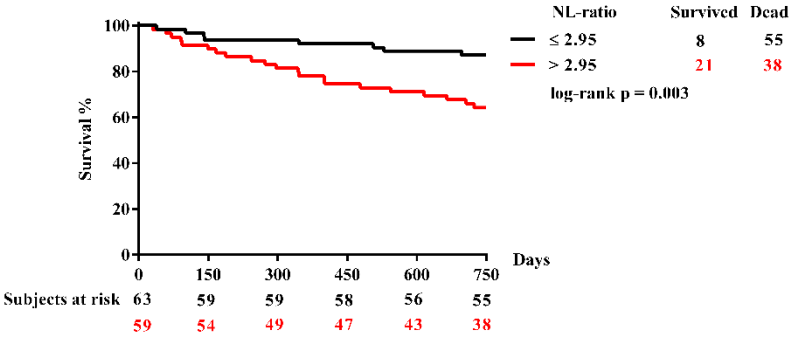

Figure 1. Influence of the baseline NL-ratio on the 2-year mortality of the patients.

We validated our results. The calibration of the prediction models were optimal (HL test: reverse remodelling p = 0.44 and mortality p = 0.79) and the performance improved (reverse remodelling: Brier score 0.22 → 0.20 and Nagelkerke's R^2 0.12→ 0.24; mortality: Brier score 0.14 → 0.12 and Nagelkerke's R^2 0.24→ 0.34). We have observed a statistically significant reclassification [NRI reverse remodelling: 0.49 (0.14-0.83), p = 0.005; NRI mortality: 0.63 (0.24-1.01), p = 0.001] and discrimination [IDI reverse remodelling: 0.04 (0.00-0.07), p = 0.02; IDI mortality 0.04 (0.00-0.08), p = 0.02] improvement by adjusting the NL-ratio biomarker to the basic multivariable prediction model including the NT-proBNP.

The IDI = 0.04 value demonstrates that 4% of the patients could be better categorized in the risk stratification if the NL-ratio is considered. Thus, 5 patients of the 122 cases had lower baseline NT-proBNP levels than 1522 pg/mL and still did not experience reverse remodelling, or died 2 years later. On the other hand, all of these patients had an elevated NL-ratio.

4.3. RDW subpopulation

4.3.1. Changes in laboratory parameters 6 months after CRT implantation

The RDW remained statistically unaltered [13.6% (13.0-14.6) vs. 13.4% (13.0-14.2), p = 0.56] 6 months after CRT implantation.

4.3.2. Prediction of the 6-month reverse remodelling

A total of 123 patients were analyzed for reverse remodelling. The responders to CRT (n = 61, 50%) were younger and presented with higher left ventricular volumes, a better NYHA functional status, and more frequent MRI usage. Increasing baseline levels of RDW [OR = 1.52 (1.01-2.29), p = 0.04; per 1 standard deviation increase], NT-proBNP [OR = 2.00 (1.19-3.38), p = 0.009] and creatinine [OR = 1.56 (1.04-2.34), p = 0.02] predicted the lack of reverse remodelling in univariate logistic regression analysis. Hematocrit concentrations were not associated with reverse remodelling [OR = 0.70 (0.49-1.02), p = 0.06]. In the multivariable analysis involving the significant factors of the univariate models, only NT-proBNP [OR = 2.67 (1.06-6.69), p = 0.03] remained statistically significant laboratory predictor of a nonresponse [RDW OR = 1.01 (0.62-0.63), p = 0.95].

4.3.3. Prediction of the 5-year mortality

Up to a median follow-up of 1799 days (maximum 2181 days), 57 (42%) patients died. Those patients survived longer, who displayed LBBB morphology in the ECG or were on beta-blocker therapy. Increasing baseline levels of RDW [HR = 1.48 (1.25-1.75), p < 0.0001; per 1 standard deviation increase], NT-proBNP [HR = 1.43 (1.19-1.73), p < 0.0001], and serum creatinine [HR = 1.42 (1.18-1.71), p < 0.0001] worsened the long-term survival chances. Elevated hematocrit fractions improved the survival [HR = 0.70 (0.53-0.92), p = 0.01]. Receiver operating characteristic analysis was performed to obtain optimal cut-off values. Each laboratory parameter was dichotomized so as to reach a sensitivity of 79% (66– 88) in mortality prediction, which we considered clinically meaningful. In this way, the individual specificity values were different, but the sensitivity was the same in all cases, making the cut-off selection more objective and comparable.

Patients before CRT were subject to up to a 3-fold increased 5-year mortality risk, with RDW levels > l3.35% [HR = 3.20 (1.69-6.06), p = 0.0002; specificity = 53% (42-65)], NT-proBNP levels > 1975 pg/mL [HR = 2.71 (1.43-5.14), p = 0.001; specificity = 48% (37-60)] and serum creatinine > 88.5 μmol/L [HR = 2.80 (1.45-5.42), p = 0.001; specificity = 47% (35-59)]. A hematocrit < 44% [HR = 1.59 (0.84–3.00), p = 0.15; specificity = 34% (24-45)] did not predict mortality statistically significant.

We performed multivariable Cox regression analyses and set up a basic multivariable prediction model with significant clinical (LBBB and beta-blocker therapy) and laboratory parameters (serum creatinine > 88.5 μmol/L). After separate adjustment to the basic model, both the RDW > 13.35% [HR = 2.81 (1.45-5.44), p = 0.002] and NT-proBNP > 1975 pg/mL [HR = 2.19 (1.13–4.23), p = 0.01] predicted the 5-year mortality. In the final model including all variables, the RDW remained significant [HR = 2.49 (1.27–4.86), p = 0.008], whereas the NT-proBNP lost its predictive value [HR = 1.18 (0.93–3.51), p = 0.07].

We validated our results. The calibration of the prediction models were optimal (final model HL test: p = 0.40) and the performance improved (Brier score 0.20 → 0.18 and Nagelkerke's R^2 0.21→ 0.30). In the final model, we have observed a statistically significant reclassification [NRI: 0.64 (0.33-0.95), p < 0.0001] and discrimination [IDI: 0.03 (0.00-0.07), p = 0.01] improvement by adjusting the RDW biomarker to the basic multivariable prediction model including the NT-proBNP. In contrast, there was no statistically significant reclassification [NRI: 0.14 (-0.03-0.32), p = 0.11] and discrimination [IDI: 0.01 (-0.01-0.03), p = 0.20] improvement by adjusting the NT-proBNP biomarker to the basic multivariable prediction model including the RDW.

The IDI = 0.03 value demonstrates that 3% of the patients could be better categorized in the risk stratification if the RDW is considered. Thus, 4 patients of the 134 cases had low NT-proBNP levels before CRT, but high RDW and died 5 years later.

4.4. Complement components subpopulation

4.4.1. Changes in laboratory parameters 6 months after CRT implantation

A statistically significant reduction was observed in the C3a [212.5 (148.2-283.6) vs. 153 (119.8-218.3) ng/mL, p < 0.0001] and in the sC5b-9 levels [296.9 (234.2-358.8) vs. 255.1 (210.1-319.0) ng/mL, p = 0.0006]. In contrast, there was no relevant difference in the total C3 protein levels [1.43 (1.26-1.61) vs. 1.38 (1.23-1.57) g/L, p = 0.57].

4.4.2. Prediction of the 6-month reverse remodelling

The CRT exerted reverse remodelling in 52% of the cases (n = 65), defined as at least 15% decrease in LVESV. These patients showed lower baseline C3a levels [186.4 (135.9-232.2) vs. 226.6 (157.9-289.8) ng/mL, p = 0.02], but there was no statistically significant difference as concerns the total C3 levels [1.42 (1.29-1.59) vs. 1.39 (1.26-1.61) g/L, p = 0.54] and sC5b-9 [278.3 (225.9-364.5) vs. 315.0 (265.4-363.7) ng/mL, p = 0.15, respectively]. We observed a statistically significant positive correlation between the C3a and the LVESV changes [r = 0.19 (0.01-0.38), p = 0.04], but neither the total C3 [r = -0.06 (-0.26-0.14), p = 0.53] nor the sC5b-9 changes [r = -0.09 (-0.29-0.11), p = 0.35] correlated significantly with the echocardiographic changes.

The univariate logistic regression analysis demonstrated that younger age, female gender, wider QRS and lower NT-proBNP levels were associated with the echocardiographic response following CRT. None of the complement components predicted statistically significant reverse remodelling. Using a strict criteria for determining clinical responsiveness to CRT (presence of at least 15% decrease of LVESV and at least 1 improvement in NYHA class and lack of heart failure hospitalization in the first 6 months), good clinical response was observed in 31% of the cases (n = 39). Responders had lower baseline C3a levels compared with non-responders [186.4 (118.8-247.9) vs. 217.4 (156.6-299.9) ng/mL, p = 0.01], but there was no statistically significant difference in the total C3 levels [1.40 (1.25-1.63) vs. 1.43 (1.28-1.58) g/L, p = 0.64] and sC5b 9 [298.4 (223.0-399.8) vs. 295.7 (246.5-362.0) ng/mL, p = 0.81, respectively].

4.4.3. Prediction of the 5-year mortality

After a median follow-up period of 1803 days, 51 (40%) patients died. The univariate Cox regression analysis revealed that patients survived better with LBBB morphology in ECG and with beta-blocker therapy. On the other hand, elevated NT-proBNP and C3a levels worsened the survival chances. ROC analysis was performed to obtain optimal cut-off values. Each laboratory parameter was dichotomized so as to reach a sensitivity of 90% (79–97) in mortality prediction, which we considered clinically meaningful. In this way, the individual specificity values were different, but the sensitivity was the same in all cases, making the cut-off selection more objective and comparable. C3a levels exceeding 165 ng/mL (log-rank p < 0.0001) and NT-

proBNP levels over 1520 pg/mL (log-rank p = 0.0004) statistically significant worsened survival chances.

We performed multivariable Cox regression analyses and set up a multivariable basic prediction model with significant clinical (LBBB and beta-blocker therapy) and laboratory parameters (NT-proBNP). After adjustment of C3a to this model, both the C3a >165 ng/mL [HR = 4.21 (1.65–10.72), p = 0.003] and NT-proBNP > 1520 pg/mL [HR = 3.39 (1.31–8.74), p = 0.01] predicted the 5-year mortality.

We validated our results. The calibration of the prediction models were optimal (final model HL test: p = 0.95) and the performance improved (Brier score 0.20 → 0.18 and Nagelkerke's R^2 0.21→ 0.31). In the final model, we have observed a statistically significant reclassification [NRI: 0.71 (0.43-0.98), p < 0.0001] and discrimination [IDI: 0.08 (0.03-0.12), p = 0.0002] improvement by adjusting the C3a biomarker to the basic multivariable prediction model including the NT-proBNP.

The IDI = 0.08 value demonstrates that 8% of the patients could be better categorized in the risk stratification if the RDW is considered. Thus, 10 patients of the 126 cases had low NT-proBNP levels before CRT, but high C3a and died 5 years later.

4.5. Additional calculations

We performed additional calculations to link the results derived from the analyses of NL-ratio, RDW and complement components and investigate their co-effect in the prediction. In this linked population (n = 114), the univariate Cox regression analysis revealed that 5-year mortality (n = 48) was statistically significant associated with less beta-blocker therapy and non-LBBB morphology in ECG and consequently these parameters comprised the basic model of the multivariable analysis. In the final multivariable we adjusted NT-proBNP, RDW, NL-ratio and C3a to the basic model and both NT-proBNP [HR = 1.32 (1.06-1.64), p = 0.01; per 1 standard deviation increase], RDW [HR = 1.24 (1.00-1.53), p = 0.04] and NL-ratio [HR = 1.37 (1.08-1.74), p = 0.008] remained statistically significant predictor of the 5-year mortality, whereas C3a lost its predictive capacity [HR = 1.20 (0.93-1.55), p = 0.15].

5. Conclusions

This is the first study that demonstrates how CRT affects routinely-used biomarkers and the correlations between the echocardiographic and laboratory changes. Our group presented the strengths and limitations of traditional and novel, specific and non-specific biomarkers, and provided an overview on the possibilities of biomarker research in CRT. We tested biomarkers that are widely available in clinical practice: C3a could be measured via commercially available ELISA kits and modern, automated blood count systems can easily determine the red blood cell distribution width or the fractions of the lymphocytes and the neutrophils with great precision and most importantly with low cost. The main strength of our study is that nearly all patients have blood count data in the clinical practice, RDW is directly displayed in the lab results, whereas the NL-ratio could be calculated (or at least estimated) with a little effort. The key message of our research is that an increased RDW or NL-ratio should draw attention to a possible adverse outcome in chronic heart failure patients undergoing CRT.

Our main results:

1. The routine laboratory measurements of chronic heart failure patients presenting for CRT suggest cardiac congestion, tissue damage and steady-state inflammation, impaired renal and hepatobiliary perfusion and metabolic alterations. CRT results in echocardiographic reverse remodelling, which is followed by beneficial biochemical changes.

2. The NL-ratio is elevated in chronic heart failure patients as compared to healthy subjects. CRT decreases the NL-ratio 6 months after implantation. Patients who experience 6-month reverse remodelling have lower NL-ratio at baseline. An increased NL-ratio before CRT implantation predicts the lack of 6-month reverse remodelling and 2-year mortality independently of NT-proBNP and other factors.

3. The RDW levels do not change statistically significant 6 months after CRT implantation. Increasing levels of RDW before CRT predict the lack of 6-month reverse remodelling, but not independently of NT-proBNP or other factors. Increasing levels of RDW before CRT predict the 5-year

mortality of CRT patients independently of NT-proBNP and other factors. Reclassification analyses revealed that RDW is superior to NT-proBNP from the aspect of long-term mortality prediction.

4. CRT decreases the levels of activated complement components (C3a, sC5b9) in chronic heart failure patients, but does not influence the total complement concentration (C3). Patients who experience 6-month reverse remodelling have lower C3a at baseline. The C3a changes follow echocardiographic improvements, but C3a does not predict reverse remodelling. Increasing levels of C3a before CRT predict the 5-year mortality of CRT patients independently of NT-proBNP and other factors.

5. Evaluation of NT-proBNP, NL-ratio, RDW and C3a biomarkers in linked multivariable models of the 5-year mortality prediction shows that NT-proBNP, NL-ratio, and RDW remain statistically significant factors, while C3a lost its predictive capacity.

6. Bibliography of the candidate's publications

Sum of impact factors of publications related to the dissertation: 9.528.

Boros AM, Perge P, Nagy KV, Apor A, Bagyura Z, Zima E, Molnar L, Tahin T, Becker D, Geller L, Merkely B, Szeplaki G. (2017) Routine laboratory parameters in cardiac resynchronization therapy. Interv Med Appl Sci, 9: 1-8.

Boros AM, Szeplaki G, Perge P, Jenei Z, Bagyura Z, Zima E, Molnar L, Apor A, Becker D, Geller L, Prohaszka Z, Merkely B. (2015) The ratio of the neutrophil leucocytes to the lymphocytes predicts the outcome after cardiac resynchronization therapy. Europace, 18: 747-54. Impact factor: 4.521.

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, 7304538: 13. Impact factor: 2.348.

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Sum of impact factors of publications not related to the dissertation: 7.578.

Nagy VK, Szeplaki G, Apor A, Kutyifa V, Kovacs A, Kosztin A, Becker D, Boros AM, Geller L, Merkely B. (2015) Role of Right Ventricular Global Longitudinal Strain in Predicting Early and Long-Term Mortality in Cardiac Resynchronization Therapy Patients. PLoS One, 10: e0143907. Impact factor: 3.057.

Merkely B, Kosztin A, Roka A, Geller L, Zima E, Kovacs A, Boros AM, Klein H, Wranicz JK, Hindricks G, Clemens M, Duray GZ, Moss AJ, Goldenberg I, Kutyifa V. (2017) Rationale and design of the BUDAPEST-CRT Upgrade Study: a prospective, randomized, multicentre clinical trial. Europace, In Press. Impact factor: 4.521.