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Impact of CT-apelin and NT-proBNP on identifying non-responders to cardiac resynchronization therapy

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ABSTRACT

Context: Assessment of response to cardiac resynchronization therapy (CRT) is essential.

Objective: To assess the predictive value of CT-apelin together with NT-proBNP in patients undergoing CRT.

Methods: Serum CT-apelin and NT-proBNP were measured by ELISA before, and six months after CRT. Primary endpoint was non-response (<4% increase in LVEF) after six months.

Results: From 81 patients, 15 proved to be non-responders. Six-month CT-apelin was superior compared to NT-proBNP in identifying non-responders by multivariate ROC (CT-apelin: $p = 0.01$, NT-proBNP: $p = 0.13$) and by logistic regression (CT-apelin: $p = 0.01$, NT-proBNP: $p = 0.41$) analyses.

Conclusion: Six-month CT-apelin might be a valuable novel biomarker in identifying non-responders to CRT that was superior to NT-proBNP.

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Context


Cardiac resynchronization therapy (CRT) improves left ventricular function, reduces symptoms and all-cause mortality in patients with symptomatic systolic heart failure and left bundle branch block (Bristow et al., 2004; Cleland et al., 2005; Goldenberg et al., 2014; Moss et al., 2009). However, a significant number of heart failure patients fail to develop reverse remodeling after CRT implantation (Goldenberg et al., 2011). The rate of non-response varies largely depending on the definitions used (32–91%) (Fornwalt et al., 2010), but non-responder patients were consistently shown to have significantly higher risk for mortality and rehospitalization (Goldenberg et al., 2011). In order to predict and prevent non-response, several trials investigated optimal patient selection and proper risk stratification (Chan et al., 2010; Goldenberg et al., 2011; Shanks et al., 2011). Based on these, left bundle branch block (LBBB) morphology and QRS duration seem to play key roles in success (Zareba et al., 2011). However, it may be desirable not only to predict, but also to accurately identify non-responders after CRT. Although echocardiographic control is still the gold standard to evaluate response to CRT after 3–6 months of implantation, it has a large inter-observer variability with the need for more objective markers of responsiveness (Bellenger et al., 2000; Blondheim et al., 2010).

Biomarkers are often used to obtain prognostic information for heart failure patients and to help guiding treatment during medical management (Maisel, 2011; Maisel & Choudhary, 2012). NT-proBNP is the gold standard marker to assess the severity of heart failure, determine prognosis and tailor medical therapy (Berger et al., 2009; Brenyo et al., 2013). However, prior studies failed to confirm its role as an independent predictor of response to CRT (Anand et al., 2003; Masson et al., 2008).

Apelin, the endogenous ligand for the G protein-coupled apelin receptor, is emerging as an important regulator of the cardiovascular homeostasis (O'Carroll et al., 2013). The 77-amino acid preproapelin is cleaved to shorter peptides, such as apelin-36, apelin-17, apelin-13, apelin-12, and the post-translationally modified (Pyr¹)apelin-13. These C-terminal apelin fragments (CT-apelin) are all agonists of the apelin receptor, but binding affinity and biological efficacy differ from isoform to isoform. In line with the abundant expression of apelin and its cognate receptor throughout the cardiovascular system, the peptide is a major autocrine/paracrine regulator of vascular tone (O'Carroll et al., 2013), volume regulation, myocardial contractility and glucose–lipid metabolism (Ashley et al., 2005; Farkasfalvi et al., 2007; Japp & Newby, 2008; Perjes et al., 2014; Szokodi et al., 2002). Moreover, apelin induces endothelium-dependent

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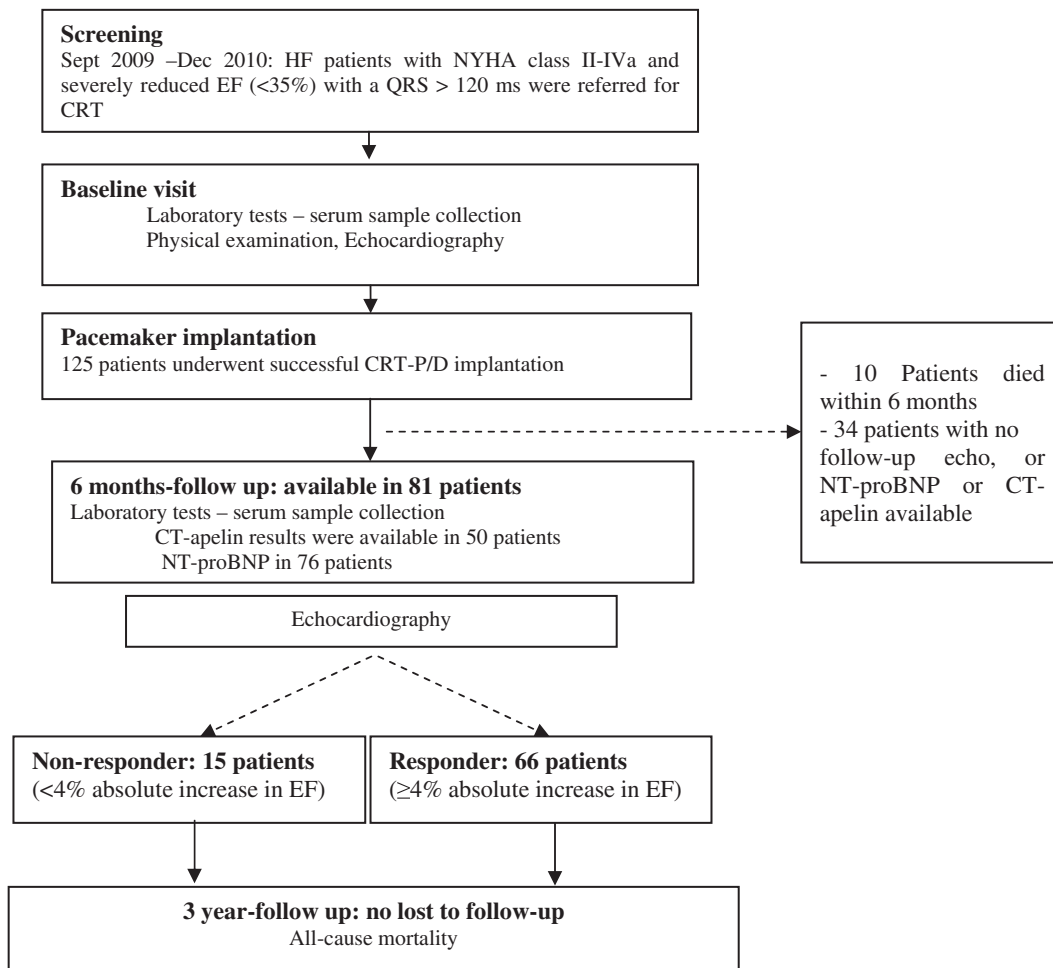


Figure 1. Flowchart of patient enrollment and follow up.

vasodilation on both arterial and venous side of the circulation (Tatemoto et al., 2001), thereby reducing cardiac preload and afterload *in vivo* (Ashley et al., 2005). The beneficial hemodynamic effects of the peptide are preserved in experimental (Berry et al., 2004) and human chronic heart failure (Barnes et al., 2013; Japp et al., 2010), which is reflected also in a direct anti-remodeling effect, in reduced cardiac hypertrophy versus interstitial fibrosis, and promoted neo-angiogenesis (Kuba et al., 2007; Siddiquee et al., 2011; Wang et al., 2013). Moreover one of the most potent endogenous positive inotropic agent (Perjes et al., 2014).

However, the role of apelin in heart failure is still unclear as changes of plasma levels are controversial in humans during the progression of heart failure (Chen et al., 2003; Chong et al., 2006; Foldes et al., 2003; Miettinen et al., 2007). In addition, no data is available on its value in predicting or evaluating the response to CRT. Therefore, we aimed to determine the clinical value of CT-apelin and NT-proBNP in identifying non-responders after CRT.

Objectives

Patient recruitment and follow-up:

A single-center, prospective, observational, cohort study was performed in patients undergoing CRT due to severe

chronic systolic heart failure. In line with current practice guidelines, inclusion criteria included ejection fraction under 35%, prolonged QRS duration (LBBB: QRS >120 ms, non-LBBB: QRS >150 ms) and symptoms of heart failure (NYHA II–IVa functional class) despite optimal pharmacological treatment (Brignole et al., 2013). Exclusion criteria included severely reduced life expectancy (<1 year), active malignant state and complications or failures during CRT implantation. In addition, patients died before the first follow-up visit at six months were also censored due to the lack of ability to classify them according to response criteria. During CRT, septal (right ventricular lead) and posterolateral/lateral (left ventricular lead) positions were preferred to reach a higher responder rate.

Detailed laboratory, echocardiographic and physical examinations were performed at the time of implantation and six months after CRT. Beyond regular outpatient visits, patients were contacted via telephone to obtain vital information at three years after CRT implantation. In addition, a national database of vital records was used to track fatal events (Figure 1). Prior to utilization in our database all relevant patient data was anonymized and encoded. The study was approved by the Institutional Scientific Ethics Committee and all patients provided written informed consents for enrolment.

Materials and methods

Endpoints

The primary endpoint of the study was non-response to CRT defined as an absolute increase of less than 4% in ejection fraction (Rickard et al., 2014) at six months, compared to baseline measurements. Key secondary endpoint included all-cause mortality during three years follow-up.

Biomarker measurements

Human CT-apelin was measured by using C-terminus Enzyme Immunoassay competitive ELISA method (RayBiotech, Inc., Norcross, GA) which is designed to target the C-terminus of the 77-aa apelin peptide. The test detects all active forms of apelin fragments including apelin-13, -31, -28 and apelin-36. NT-proBNP was measured with Cobas proBNP II kit (Roche Diagnostics GmbH, Mannheim, Germany). Samples were collected at baseline and 6 months after CRT. Serum samples were stored at -80°C after collection and were assayed later when sample collection was completed.

Echocardiography

Detailed echocardiographic measurements were performed both at baseline and 6 months after CRT. To exclude inter-observer variability, ejection fraction was assessed with biplane Simpson method by the same experienced investigator at both time points (Philips iE33 system, Philips Healthcare, Best, The Netherlands).

Statistical analysis

Continuous variables with normal distributions are expressed as mean \pm SD, while those with non-normal distributions as medians with interquartile range (IQR). Categorical variables are summarized with frequencies and percentages (n , %). Baseline clinical characteristics were compared between the responder and non-responder groups using unpaired t -test for normally distributed continuous variables, the Mann-Whitney for non-normally distributed variables, while χ^2 -test or Fisher exact test was used for dichotomous variables, as appropriate. Univariate and multivariable receiver-operator characteristic (ROC) curve analyses were used to determine the discriminatory capacity of CT-apelin and NT-proBNP on non-response after CRT. First, univariate ROC test was used to determine the area under curve (AUC) and p values. In case of a significant p value, an optimal cutoff was determined for the continuous variable based on maximal sensitivity and specificity that best discriminated between responder and non-responder patients. Using these cutoffs, patients were separated to low and high biomarker level groups for logistic regression analyses. Multivariate logistic regressions were performed with variables showing a p value less than 0.05 in univariate analyses. Time-to-event data is presented by Kaplan-Meier curves. Event rates represent Kaplan-Meier estimates. Unadjusted hazard ratios (HR) with 95 confidence intervals (95%CI) were determined for mortality in Cox

proportional hazards models. Adjusted HR was calculated in forward stepwise Cox proportional model, including known predictors of responsiveness and/or mortality after CRT (such as age, QRS duration, LBBB morphology, female gender, ischemic etiology). A two-sided p value of <0.05 was considered statistically significant. Analyses were carried out with Graph Pad 6.0 and SPSS v9 (SPSS Inc., Chicago, IL).

Results

Baseline patient characteristics

Between September 2009 and December 2010, 81 patients underwent successful CRT implantation and were included in the study. Mean age of recruited patients was 64.9 ± 10.5 years, with a mean ejection fraction of $28.5 \pm 6.5\%$. Seventy-five percent of the patients were in NYHA class III functional state and 59% had ischemic etiology before CRT implantation (Table 1).

Response and prognosis

During the mean follow-up of 795 days, seven (9%) patients died. Based on the pre-defined classification of response, 15 (18.5%) patients proved to be non-responders. Baseline clinical characteristics, medical therapy and echocardiographic findings were similar between responders and non-responders (Tables 2 and 3). In line with the definition of response, left ventricular volumes significantly decreased (ESV: 179.1 ± 64.9 versus 117.9 ± 58.9 , $p < 0.0001$, EDV: 248.6 ± 80.2 versus 196.7 ± 77.5 , $p < 0.0001$) and left ventricular function significantly improved (EF: 28.1 ± 6.0 versus 41.3 ± 7.9) in responder patients after CRT implantation (Tables 2), while these parameters remained unchanged in the non-responder group. (Tables 2) According to Cox-regression analysis, non-responders had an almost four-fold higher risk for mortality compared with responders (HR: 3.75; 95%CI: 1.00–13.97; $p = 0.049$) (Figure 2).

This impact on mortality persisted also in the multivariate model, with non-response to CRT prevailing as an independent predictor of mortality (adjusted HR: 4.54, 95%CI: 1.14–18.15, $p = 0.03$).

Biomarkers to identify non-responders

At baseline, serum CT-apelin and NT-proBNP levels were similar in both responders and non-responder patients ($p = 0.74$) (Table 1) and ROC testing showed that these parameters are not predictors of non-response (apelin: AUC 0.48; 95%CI: 0.29–0.70; $p = 0.87$, NT-proBNP: AUC 0.53; 95%CI: 0.37–0.70; $p = 0.73$).

At six months, serum CT-apelin significantly decreased in responders (from 549.5 ng/ml [IQR: 279.0–868.8] to 211.0 ng/ml [IQR: 113.8–416.8]; $p < 0.0001$), while it remained unchanged in non-responder patients (from 472.5 ng/ml [IQR: 307.8–700.3] to 541.0 ng/ml [IQR: 278.3–831.0]; $p = 0.80$) (Figure 3). Similarly, NT-proBNP levels significantly decreased in responders at six months (median: 2561 pg/ml, IQR: 1173–4616 to 1253 pg/ml, IQR: 516–2519; $p = 0.007$), while it

Table 1. Baseline clinical variables, prior medical history, echocardiographic measurements, serum peptides and medical therapy in the responder and non-responder patients.

Baseline clinical variables	All patients (n = 81)	Responders (n = 66)	Non-responders (n = 15)	p Value
Age (years, mean ± SD)	64.9 ± 10.49	64.1 ± 10.8	68.5 ± 8.4	0.14
Gender (female, n, %)	15 (18.5%)	14 (21%)	1 (7%)	0.28
Ischemic etiology (n, %)	48 (59%)	39 (59%)	9 (60%)	1.00
NYHA II. st (n, %)	11 (14%)	9 (14%)	2 (13%)	1.00
NYHA III. st (n, %)	61 (75%)	49 (74%)	12 (80%)	0.75
NYHA IV. st (n, %)	9 (11%)	8 (12%)	1 (7%)	1.00
QRS (ms, mean ± SD)	167.7 ± 29.8	166.6 ± 28.8	172.0 ± 34.3	0.53
Typical LBBB morphology (n, %)	70 (86%)	57 (86%)	13 (87%)	1.00
Not typical LBBB (n, %)	11 (14%)	9 (14%)	2 (13%)	1.00
6 minutes walk test (m, mean ± SD)	311.4 ± 117.1	307.3 ± 127.6	329.2 ± 54.1	0.56
RR systolic (mmHg, mean ± SD)	120.4 ± 18.8	121.1 ± 17.4	117.5 ± 24.8	0.51
RR diastolic (mmHg, mean ± SD)	76.2 ± 10.7	76.9 ± 10.2	73.1 ± 12.3	0.21
Heart rate (min ⁻¹ , mean ± SD)	75.6 ± 14.5	75.6 ± 14.1	75.3 ± 16.5	0.93
Atrial fibrillation (n, %)	20 (25%)	14 (21%)	6 (40%)	0.18
Body mass index (BMI; med, IQR)	27.0 (24/30)	27.0 (24/30)	29.0 (26/31)	0.16
Medical history				
Hypertension (n, %)	63 (78%)	51 (77%)	12 (80%)	1.00
Type 2 DM (n, %)	25 (31%)	22 (33%)	3 (20%)	0.37
Prior PCI (n, %)	25 (31%)	20 (30%)	5 (33%)	1.00
Prior CABG (n, %)	14 (17%)	11 (17%)	3 (20%)	0.72
Prior stroke (n, %)	8 (10%)	6 (9%)	2 (13%)	0.64
Prior COPD (n, %)	10 (12%)	8 (12%)	2 (13%)	1.00
Prior dyslipidaemia (n, %)	28 (35%)	20 (30%)	8 (53%)	0.13
Prior major arrhythmia – VF (n, %)	3 (4%)	2 (3%)	1 (7%)	0.46
Prior ICD implantation (n, %)	7 (9%)	4 (6%)	3 (20%)	0.11
Echocardiographic parameters				
LV ejection fraction (Simpson%, mean ± SD)	28.5 ± 6.5	28.1 ± 6.0	30.4 ± 8.2	0.23
LV end-systolic volume (ml, mean ± SD)	183.5 ± 63.3	179.1 ± 64.9	203.0 ± 51.5	0.22
LV end-diastolic volume (ml, mean ± SD)	254.7 ± 79.1	248.6 ± 80.2	281.5 ± 67.9	0.18
Serum peptides				
NT-proBNP (pg/ml; med, IQR)	2573 (1207/4611)	2561 (1173/4616)	3126 (1238/4492)	0.61
CT-apelin (ng/ml; med, IQR)	512.0 (288.3/808.8)	549.5 (279.0/868.8)	472.5 (307.8/700.3)	0.74
Baseline medical therapy (n, %)				
Beta blocker (n, %)	74 (91%)	60 (91%)	14 (93%)	1.00
ACE inhibitor or ARB (n, %)	77 (95%)	63 (96%)	14 (93%)	0.57
Spirolactone (n, %)	56 (69%)	46 (70%)	10 (67%)	1.00
Eplerenone (n, %)	7 (9%)	5 (8%)	2 (13%)	0.61
Furosemide (n, %)	62 (77%)	49 (74%)	13 (87%)	0.50
Hydrochlorotiazide (n, %)	9 (11%)	7 (11%)	2 (13%)	0.67
Hydralazine (n, %)	5 (6%)	4 (6%)	1 (7%)	1.00
Digoxin (n, %)	23 (28%)	20 (30%)	3 (20%)	0.54
Amiodarone (n, %)	23 (28%)	19 (29%)	4 (27%)	1.00
Statin (n, %)	50 (62%)	38 (58%)	12 (80%)	0.15
Aspirin (n, %)	38 (47%)	34 (52%)	4 (27%)	0.10
Clopidogrel (n, %)	20 (25%)	17 (26%)	3 (20%)	0.75
Oral anticoagulant therapy (n, %)	26 (32%)	19 (29%)	7 (47%)	0.22

LBBB: left bundle branch block; COPD: chronic obstructive pulmonary disease; PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; VF: ventricular fibrillation; ACE: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker.

Table 2. Changes in echocardiographic parameters compared to baseline six months after CRT.

Responder patients	Baseline	Follow up	p value
LV ejection fraction (Simpson%, mean ± SD)	28.1 ± 6.0	41.3 ± 7.9	<0.0001***
LV end-systolic volume (ml, mean ± SD)	179.1 ± 64.9	117.9 ± 58.9	<0.0001***
LV end-diastolic volume (ml, mean ± SD)	248.6 ± 80.2	196.7 ± 77.5	<0.0001**
Non-responder patients			
LV ejection fraction (Simpson%, mean ± SD)	30.4 ± 8.2	29.3 ± 7.1	0.34
LV end-systolic volume (ml, mean ± SD)	203.0 ± 51.5	194.8 ± 46.9	0.38
LV end-diastolic volume (ml, mean ± SD)	281.5 ± 67.9	271.6 ± 56.1	0.43

p < 0.01, *p < 0.001.

Table 3. Changes in serum peptide levels between responder and non-responder patients after CRT implantation.

Responder patients	Baseline	Follow up	p value
CT-apelin (ng/ml, med, IQR)	549.5 (279.0/868.8)	211.0 (113.8/416.8)	<0.0001***
NT-proBNP (pg/ml, med, IQR)	2561 (1173/4616)	1253 (516/2519)	0.007***
Non-responder patients			
CT-apelin (ng/ml, med, IQR)	472.5 (307.8/700.3)	541.0 (278.3/831.0)	0.80
NT-proBNP (pg/ml, med, IQR)	3126 (1238/4492)	2676 (1947/4354)	0.91

***p < 0.001.

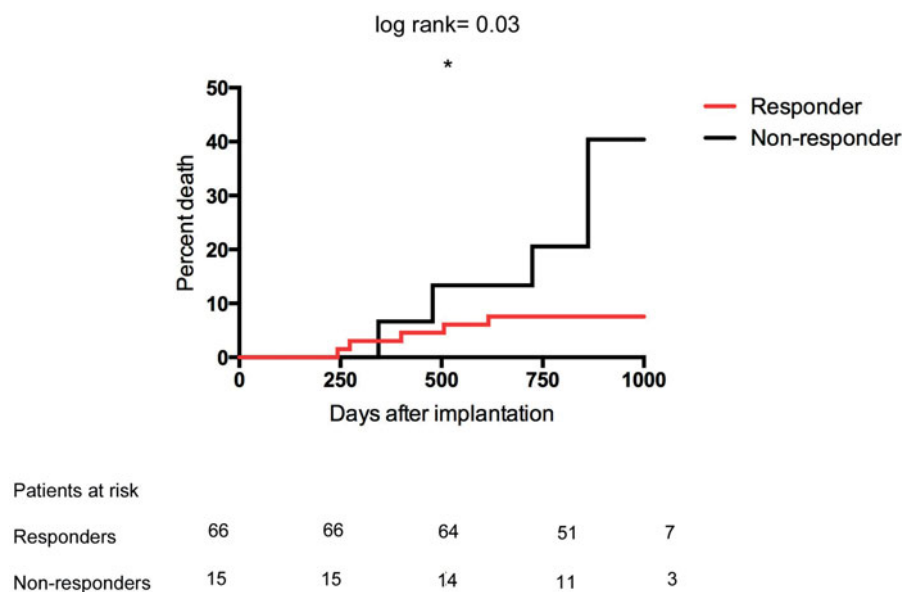


Figure 2. All-cause mortality in responder and non-responder patients to CRT.

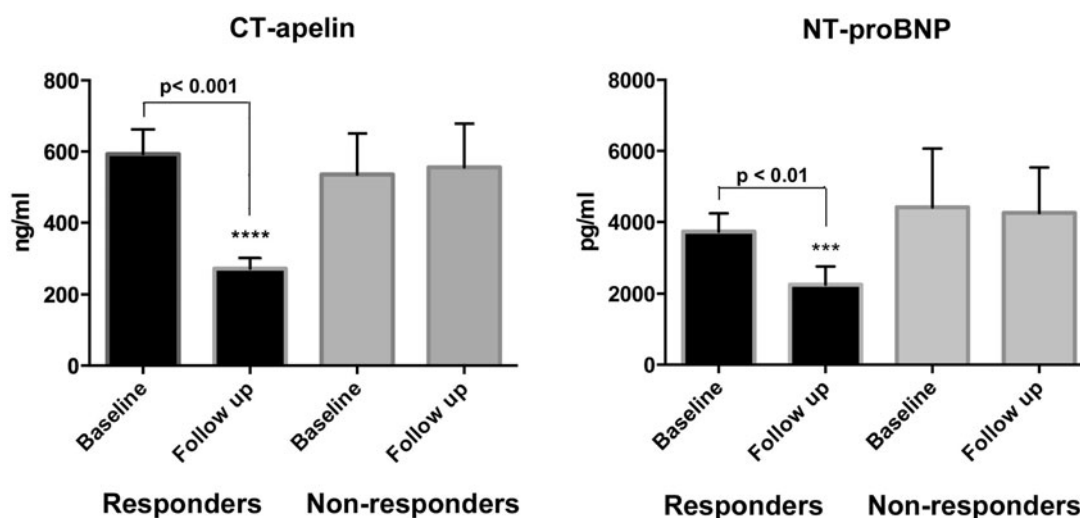


Figure 3. Changes in CT-apelin and NT-proBNP levels according to response to CRT.

remained unchanged in non-responder patients (median: 3126 pg/ml [IQR: 1238–4492] to 2676 pg/ml [IQR: 1947–4354]; $p = 0.91$).

In ROC analysis, both six-month CT-apelin and NT-proBNP levels significantly discriminated between responder and non-responder patients (CT-apelin: AUC 0.78; 95%CI: 0.59–0.97; $p < 0.01$, NT-proBNP: AUC 0.75; 95%CI: 0.62–0.88; $p = 0.005$). According to the highest sensitivity and specificity, the optimal cutoffs to diagnose non-response were 268.5 ng/ml for CT-apelin and 1348.5 pg/ml for NT-proBNP, respectively.

When patients were classified into groups according to optimal cutoff values, patients with high serum CT-apelin showed a 10 times higher odds for non-response (OR: 10.3, 95%CI: 1.16–91.43; $p = 0.04$), while higher NT-proBNP levels indicated a 16-fold odds for non-response in our patient cohort (OR: 16.0, 95%CI: 1.96–130.68; $p = 0.01$).

However, multivariate ROC testing suggested the superiority of CT-apelin over NT-proBNP (CT-apelin: AUC 0.78; 95%CI: 0.59–0.97; $p = 0.013$ versus NT-proBNP: AUC 0.67; 95%CI: 0.49–0.85; $p = 0.13$, Figure 4) that was also confirmed in multivariate logistic regression analysis (CT-apelin: $p = 0.01$, NT-proBNP: $p = 0.41$).

Discussion

Main findings of our study can be summarized as follows:

1. Our results confirm that a significant group of heart failure patients (19% in the current cohort) do not develop reverse remodeling and become non-responders to CRT, showing an elevated risk for mortality compared to good responders.

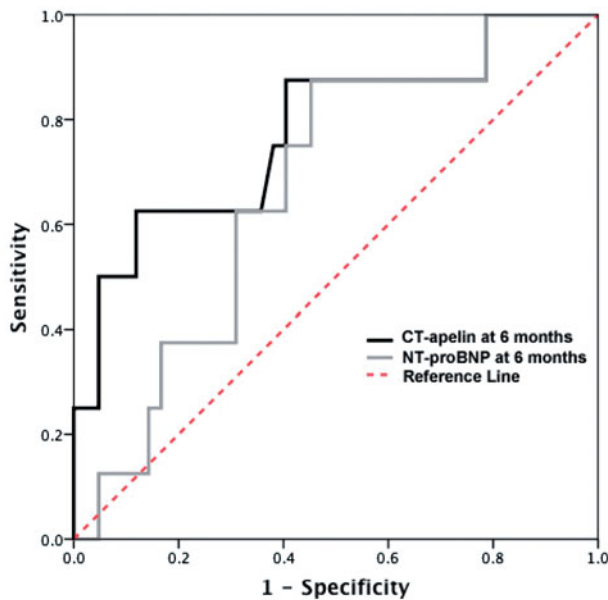


Figure 4. Receiver-operator characteristic curve analysis comparing the diagnostic performance of six-month serum CT-apelin and NT-proBNP levels on identifying non-responders to CRT.

2. Baseline levels of CT-apelin and NT-proBNP are not associated with non-response. Therefore, neither biomarkers may be considered as *predictors* of success before device implantation.
3. Six-month levels of both CT-apelin and NT-proBNP were in significant association with non-response, suggesting the possible role of such biomarkers in *identifying* non-responders. Based on multivariate models, our results suggest the superiority of CT-apelin over NT-proBNP. These biomarkers may give additional help and information to define responder status after CRT, that is in many times complicated by significant inter-observer variability of echocardiographic assessment.

CRT improves exercise capacity and reduces the risk of heart failure events that may all contribute to an improved event-free survival (Bristow et al., 2004; Cleland et al., 2005; Moss et al., 2009). However, approximately 20–40% of patients fail to develop reverse remodeling and are considered non-responders to treatment (Goldenberg et al., 2011). As such patients have in average 2–5-fold higher hazard for all-cause mortality (Goldenberg et al., 2014; Hsu et al., 2012) and heart failure events, it would be desirable to accurately identify them during follow-up.

Although NT-proBNP is a valuable biomarker to diagnose patients with heart failure and stratify them into risk categories (Hartmann et al., 2004; Masson et al., 2008), data is controversial on its possible role in evaluating the response to CRT (Brenyo et al., 2013) especially in patients with mildly symptomatic heart failure. Thus, we aimed to assess the predictive role of baseline NT-proBNP and the diagnostic value of six-month follow-up levels in identifying non-responder patients to CRT.

In our patient cohort, baseline levels were similar in responders and non-responders, but six-month NT-proBNP levels significantly decreased in responders to CRT. In line with

the biomarker data, responders showed clear echocardiographic evidence of reverse remodeling (Table 2). Similar results were found in CARE-HF trial (Fruhwald et al., 2007), where Fruhwald et al. demonstrated that CRT significantly reduces NT-proBNP levels after 3–6 months compared to optimal pharmacological treatment. The MADIT-CRT trial also suggested that baseline serum levels of NT-proBNP were not related to non-response and to echocardiographic improvements; however, follow-up levels of NT-proBNP were in significant association with the echocardiographic response to resynchronization (Brenyo et al., 2013).

In addition to NT-proBNP, a recently identified cardiac peptide, apelin has attracted considerable attention in heart failure. Although changes in plasma apelin levels during the progression of heart failure, clinical trials are controversial. In one of the largest studies including 202 patients Chong et al. found that plasma apelin-12 (also cross-reactive with apelin-13, -36 fragments) was significantly lower in patients with advanced heart failure referred for heart transplantation (Chong et al., 2006). In another study Chen et al. examined 80 patients with moderate to severe chronic heart failure compared to healthy volunteers. According to their findings, circulating apelin increases in the early stage, while in advanced heart failure it decreases to a lower level, but remains over the normal plasma range (Chen et al., 2003).

However, the role of apelin in patients after CRT is not well elucidated. To date, the only small-sized study which described changes in levels of apelin after CRT was published by Francia et al. (2007). In 14 patients undergoing device implantation, significant increase in serum apelin levels was found after nine months of resynchronization. Evidently, this low sample size did not allow the authors to compare apelin in responder and non-responder patients; the single patient considered non-responder had higher apelin level than the others.

In our patient cohort including 81 patients, responders and non-responders showed the same CT-apelin values at baseline. However, non-responders had significantly higher CT-apelin levels at six months compared to responders after CRT implantation. Likewise, patients with high CT-apelin levels had a 10-fold higher risk for non-response.

Given the potential collinearity between NT-proBNP and apelin, multivariate models were developed to determine the independent estimate of non-response. Based on such statistical models, apelin proved to be the independent biomarker in identifying non-response (Figure 4).

These results suggest that a simple measurement of apelin or NT-proBNP during follow-up of CRT implantation may help in identifying non-responders to therapy. This may be of great clinical importance due to the potential difficulties in judging relatively small absolute changes in ejection fraction during control echocardiographic measurements, as well as considering the significant inter-observer variability of the echocardiographic examination (Bellenger et al., 2000; Blondheim et al., 2010). Our results suggest that CT-apelin may be the preferred marker for classification, but further studies are needed to confirm these findings.

Conclusions

Non-responders have a significantly elevated risk for mortality after CRT implantation. Not baseline, but six-month CT-apelin and NT-proBNP levels were significant indicators of non-response to CRT. Our results suggest that a simple biomarker measurement during follow-up may help to identify non-responders who have poor outcomes after CRT implantation. Further studies using the same methodology are required to corroborate these findings in a larger patient population and to determine whether apelin may be used to alter management in patients with severe systolic heart failure.

Limitations

Our study has some certain limitations. First, this was a relatively small registry-based patient cohort with low rate of endpoint events that may result in overestimating the real predictive value of both biomarkers. Therefore, the suggested cutoff values for both biomarkers need to be validated in larger prospective studies for good and poor responders to CRT. Moreover, the low sample size might be also a reason why traditional clinical predictors of responsiveness were not identified between responders and non-responders. Notably, this is still the largest dataset among patients after CRT implantation with apelin level assessments at a relatively long (three-year) follow-up.

Second, the observed plasma levels of CT-apelin in our study were considerably higher than found in other prior studies (Foldes et al., 2003; Miettinen et al., 2007). This may be due to the various sensitivities of the assays for different apelin fragments, making it difficult to directly compare results. By using RayBiotech C-Terminus-apelin ELISA kit we have detected apelin-36, -13, -28 and -31 fragments, that might be responsible for the differences compared to other authors that usually detected only the apelin-12, -13, -36 fragments by another commercially available ELISA kit (Chen et al., 2003; Francia et al., 2007; Miettinen et al., 2007). In addition, the observed plasma apelin levels in the tested individuals were within the range of the kit provided by the test manufacturer.

Third, although baseline levels of apelin and NT-proBNP were not in relation with non-response, our results cannot determine the optimal time of biomarker sampling after CRT. We have only used six-month biomarker data, but it is possible that such associations may also exist earlier after device implantation.

Finally, the three-year rate of cardiovascular mortality may sound quite low in the present study compared to other experiences (Bristow et al., 2004; Cleland et al., 2005). However, we only included patients with successful device implantation and having six-months biomarker laboratory results available. Therefore, our results may reflect a lower-risk cohort with successful device implantation and without mortality within the first six months of CRT operation.

Disclosure statement

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References

- Anand IS, Fisher LD, Chiang YT, et al. (2003). Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT). *Circulation* 107:1278–83.
- Ashley EA, Powers J, Chen M, et al. (2005). The endogenous peptide apelin potently improves cardiac contractility and reduces cardiac loading in vivo. *Cardiovasc Res* 65:73–82.
- Barnes GD, Alam S, Carter G, et al. (2013). Sustained cardiovascular actions of APJ agonism during renin-angiotensin system activation and in patients with heart failure. *Circ Heart Fail* 6:482–91.
- Bellenger NG, Burgess MI, Ray SG, et al. (2000). Comparison of left ventricular ejection fraction and volumes in heart failure by echocardiography, radionuclide ventriculography and cardiovascular magnetic resonance; are they interchangeable? *Eur Heart J* 21:1387–96.
- Berger R, Shankar A, Fruhwald F, et al. (2009). Relationships between cardiac resynchronization therapy and N-terminal pro-brain natriuretic peptide in patients with heart failure and markers of cardiac dyssynchrony: an analysis from the Cardiac Resynchronization in Heart Failure (CARE-HF) study. *Eur Heart J* 30:2109–16.
- Berry MF, Pirolli TJ, Jayasankar V, et al. (2004). Apelin has in vivo inotropic effects on normal and failing hearts. *Circulation* 110:II187–93.
- Blondheim DS, Beerli R, Feinberg MS, et al. (2010). Reliability of visual assessment of global and segmental left ventricular function: a multicenter study by the Israeli Echocardiography Research Group. *J Am Soc Echocardiogr* 23:258–64.
- Brenyo A, Barsheshet A, Rao M, et al. (2013). Brain natriuretic peptide and cardiac resynchronization therapy in patients with mildly symptomatic heart failure. *Circ Heart Fail* 6:998–1004.
- Brignole M, Auricchio A, Baron-Esquivias G, et al. (2013). 2013 ESC Guidelines on cardiac pacing and cardiac resynchronization therapy: the Task Force on cardiac pacing and resynchronization therapy of the European Society of Cardiology (ESC). Developed in collaboration with the European Heart Rhythm Association (EHRA). *Eur Heart J* 34:2281–329.
- Bristow MR, Saxon LA, Boehmer J, et al. (2004). Cardiac-resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure. *N Engl J Med* 350:2140–50.
- Chan PS, Khumri T, Chung ES, et al. (2010). Echocardiographic dyssynchrony and health status outcomes from cardiac resynchronization therapy: insights from the PROSPECT trial. *JACC Cardiovasc Imaging* 3:451–60.
- Chen MM, Ashley EA, Deng DX, et al. (2003). Novel role for the potent endogenous inotrope apelin in human cardiac dysfunction. *Circulation* 108:1432–9.
- Chong KS, Gardner RS, Morton JJ, et al. (2006). Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. *Eur J Heart Fail* 8:355–60.
- Cleland JG, Daubert JC, Erdmann E, et al. (2005). The effect of cardiac resynchronization on morbidity and mortality in heart failure. *N Engl J Med* 352:1539–49.

- Farkasfalvi K, Stagg MA, Coppens SR, et al. (2007). Direct effects of apelin on cardiomyocyte contractility and electrophysiology. *Biochem Biophys Res Commun* 357:889–95.
- Foldes G, Horkay F, Szokodi I, et al. (2003). Circulating and cardiac levels of apelin, the novel ligand of the orphan receptor APJ, in patients with heart failure. *Biochem Biophys Res Commun* 308:480–5.
- Fornwalt BK, Sprague WW, BeDell P, et al. (2010). Agreement is poor among current criteria used to define response to cardiac resynchronization therapy. *Circulation* 121:1985–91.
- Francia P, Salvati A, Balla C, et al. (2007). Cardiac resynchronization therapy increases plasma levels of the endogenous inotrope apelin. *Eur J Heart Fail* 9:306–9.
- Fruhwald FM, Fahrleitner-Pammer A, Berger R, et al. (2007). Early and sustained effects of cardiac resynchronization therapy on N-terminal pro-B-type natriuretic peptide in patients with moderate to severe heart failure and cardiac dyssynchrony. *Eur Heart J* 28:1592–7.
- Goldenberg I, Hall WJ, Beck CA, et al. (2011). Reduction of the risk of recurring heart failure events with cardiac resynchronization therapy: MADIT-CRT (Multicenter Automatic Defibrillator Implantation Trial With Cardiac Resynchronization Therapy). *J Am Coll Cardiol* 58:729–37.
- Goldenberg I, Kutiyifa V, Klein HU, et al. (2014). Survival with cardiac-resynchronization therapy in mild heart failure. *N Engl J Med* 370:1694–701.
- Goldenberg I, Moss AJ, Hall WJ, et al. (2011). Predictors of response to cardiac resynchronization therapy in the Multicenter Automatic Defibrillator Implantation Trial with Cardiac Resynchronization Therapy (MADIT-CRT). *Circulation* 124:1527–36.
- Hartmann F, Packer M, Coats AJ, et al. (2004). Prognostic impact of plasma N-terminal pro-brain natriuretic peptide in severe chronic congestive heart failure: a substudy of the Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS) trial. *Circulation* 110:1780–6.
- Hsu JC, Solomon SD, Bourgoun M, et al. (2012). Predictors of super-response to cardiac resynchronization therapy and associated improvement in clinical outcome: the MADIT-CRT (multicenter automatic defibrillator implantation trial with cardiac resynchronization therapy) study. *J Am Coll Cardiol* 59:2366–73.
- Japp AG, Cruden NL, Barnes G, et al. (2010). Acute cardiovascular effects of apelin in humans: potential role in patients with chronic heart failure. *Circulation* 121:1818–27.
- Japp AG, Newby DE. (2008). The apelin-APJ system in heart failure: pathophysiologic relevance and therapeutic potential. *Biochem Pharmacol* 75:1882–92.
- Kuba K, Zhang L, Imai Y, et al. (2007). Impaired heart contractility in Apelin gene-deficient mice associated with aging and pressure overload. *Circ Res* 101:e32–42.
- Maisel A. (2011). Biomonitoring and biomarker-guided therapy: the next step in heart failure and biomarker research. *J Am Coll Cardiol* 58:1890–2.
- Maisel AS, Choudhary R. (2012). Biomarkers in acute heart failure – state of the art. *Nat Rev Cardiol* 9:478–90.
- Masson S, Latini R, Anand IS, et al. (2008). Prognostic value of changes in N-terminal pro-brain natriuretic peptide in Val-HeFT (Valsartan Heart Failure Trial). *J Am Coll Cardiol* 52:997–1003.
- Miettinen KH, Magga J, Vuolteenaho O, et al. (2007). Utility of plasma apelin and other indices of cardiac dysfunction in the clinical assessment of patients with dilated cardiomyopathy. *Regul Pept* 140:178–84.
- Moss AJ, Hall WJ, Cannom DS, et al. (2009). Cardiac-resynchronization therapy for the prevention of heart-failure events. *N Engl J Med* 361:1329–38.
- O'Carroll AM, Lolait SJ, Harris LE, Pope GR. (2013). The apelin receptor APJ: journey from an orphan to a multifaceted regulator of homeostasis. *J Endocrinol* 219:R13–35.
- Perjes A, Skoumal R, Tenhunen O, et al. (2014). Apelin increases cardiac contractility via protein kinase C ϵ - and extracellular signal-regulated kinase-dependent mechanisms. *PLoS One* 9:e93473.
- Rickard J, Cheng A, Spragg D, et al. (2014). Durability of the survival effect of cardiac resynchronization therapy by level of left ventricular functional improvement: fate of “nonresponders”. *Heart Rhythm* 11:412–16.
- Shanks M, Delgado V, Ng AC, et al. (2011). Clinical and echocardiographic predictors of nonresponse to cardiac resynchronization therapy. *Am Heart J* 161:552–7.
- Siddiquee K, Hampton J, Khan S, et al. (2011). Apelin protects against angiotensin II-induced cardiovascular fibrosis and decreases plasminogen activator inhibitor type-1 production. *J Hypertens* 29:724–31.
- Szokodi I, Tavi P, Foldes G, et al. (2002). Apelin, the novel endogenous ligand of the orphan receptor APJ, regulates cardiac contractility. *Circ Res* 91:434–40.
- Tatemoto K, Takayama K, Zou MX, et al. (2001). The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul Pept* 99:87–92.
- Wang W, McKinnie SM, Patel VB, et al. (2013). Loss of Apelin exacerbates myocardial infarction adverse remodeling and ischemia-reperfusion injury: therapeutic potential of synthetic Apelin analogs. *J Am Heart Assoc* 2:e000249.
- Zareba W, Klein H, Cygankiewicz I, et al. (2011). Effectiveness of cardiac resynchronization therapy by QRS Morphology in the Multicenter Automatic Defibrillator Implantation Trial-Cardiac Resynchronization Therapy (MADIT-CRT). *Circulation* 123:1061–72.