# Release characteristics of cardiac proteins after reversible or irreversible myocardial damage

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# Chapter 8

## Reverse ventricular remodeling after cardiac resynchronization therapy is associated with a reduction in serum tenascin-C and plasma matrix metalloproteinase-9 levels

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*European Journal of Heart Failure* 2007; 9 (19):1058-1063

## Abstract

**Background:** In heart failure patients, cardiac resynchronization therapy (CRT) leads to reverse ventricular remodeling. The purpose of this study was to evaluate whether changes in levels of circulating biomarkers of extracellular matrix metabolism correlate with the response to CRT.

**Methods:** Clinical parameters, left ventricular (LV) volumes, and circulating levels of tenascin-C (TNC), matrix metalloproteinase-2 (MMP-2), MMP-9, and amino-terminal propeptide of brain natriuretic peptide (NT-proBNP) were assessed in 64 patients at baseline and 6 months follow-up.

**Results:** The majority of patients (72%) showed a reduction >10% in LV end-systolic volume at follow-up, and were classified as responders to CRT. The remaining patients were classified as non-responders. In responders, a significant decrease in circulating levels of TNC (from 60±40 ng/mL to 47±30 ng/mL, $p<0.01$), MMP-9 (from 55±30 AU to 44±27 AU, $p<0.01$), and NT-proBNP (from 2106±1805 pg/mL to 1132±1289 pg/mL, $p<0.001$) were observed at follow-up; MMP-2 levels remained unchanged. In non-responders TNC, NT-proBNP, MMP-9 and MMP-2 levels remained unchanged.

**Conclusion:** At 6 months follow-up, CRT was associated with reverse LV remodeling, and a significant decrease in TNC, MMP-9, and NT-proBNP levels. This finding suggests an important role of ECM modulation in the process of reverse ventricular remodeling in patients responding to CRT.



## Keywords

Cardiac resynchronization therapy, left ventricular remodeling, extracellular matrix, tenascin-C, matrix metalloproteinases, brain natriuretic peptide.

# Introduction

Currently, cardiac resynchronization therapy (CRT) is considered a class I indication in selected patients with chronic drug-refractory heart failure[1]. Recently conducted large randomized trials have clearly demonstrated the beneficial effects of CRT on clinical symptoms and left ventricular (LV) systolic function[2;3]. In addition, CRT resulted in less heart failure-related hospitalizations and improved patient survival[4;5]. Recently, Yu et al.[6] reported that the improvement in survival after CRT was strongly related to reverse LV remodeling at mid-term follow-up.

At present, the precise mechanisms underlying reverse LV remodeling following CRT are not completely understood. Recent data indicate that LV remodeling, during development of heart failure, is accompanied by changes in structure and composition of the myocardial extracellular matrix (ECM)[7]. Several proteins related to ECM metabolism have been studied for their role as biochemical markers predicting LV remodeling, including matrix metalloproteinase (MMPs)[8;9], tenascin-C (TNC)[10], and amino-terminal propeptide of brain natriuretic peptide (NT-proBNP) levels[11]. However, the role of these biochemical markers in the process of *reverse* LV remodeling following CRT has not been studied. Therefore, in the present study, we evaluated the value of serum or plasma levels of TNC, MMP-2, MMP-9, and NT-proBNP in patients with heart failure undergoing CRT implantation as biochemical markers of reverse LV remodeling.

# Materials and Methods

### Patients

Sixty-four patients with heart failure, scheduled for implantation of a CRT device, were included in the study. The selection criteria for CRT included: moderate-to-severe heart failure (NYHA class III-IV), LV ejection fraction ≤35%, and QRS duration >120 ms. Patients with a recent myocardial infarction (<3 months) or decompensated heart failure were excluded. Before CRT implantation (baseline) clinical status was assessed. In addition, 2-dimensional echocardiography was performed to determine LV volumes and LV ejection fraction. All clinical and echocardiographic parameters were re-assessed at 6 months follow-up.

### Clinical evaluation

Evaluation of clinical status included assessment of NYHA functional class and 6-minute hall-walk test.

### Echocardiography

Echocardiography was conducted as previously described[12]. Imaging was performed using a commercially available system (Vingmed system Seven, General Electric-Vingmed, Milwaukee, Wisconsin, USA). Patients were imaged in the left lateral decubitus position, using a 3.5 MHz transducer, at a depth of 16 cm in the parasternal and apical views (standard long axis, 2- and 4-chamber images). LV end-systolic and end-diastolic volumes (LVESV and LVEDV) and LV ejection fraction (LVEF) were calculated from the conventional apical 2- and 4-chamber images, using the biplane Simpson's technique[13].

Data were analyzed using commercial software (Echopac version 5.0.1, General Electric Vingmed). Echocardiographic data were analyzed by 2 independent observers, blinded to all other patient data. Based on recent data[6], patients with a reduction >10% in LVESV at 6 months follow-up were considered responders to CRT, whereas patients with a reduction ≤10% in LVESV, as well as patients with increased LVESV at 6 months follow-up, were classified as non-responders.

### Pacemaker implantation

The LV pacing lead was inserted transvenously via the subclavian route. A coronary sinus venogram was obtained using a balloon catheter. LV pacing lead was inserted through the coronary sinus with the help of an 8 Fr-guiding catheter, and positioned as far as possible in the venous system, preferably in a (postero-) lateral vein. The right atrial and right ventricular leads were positioned conventionally. CRT-device and lead implantation were successful in all patients without major complications (Contak TR or Contak Renewal TR2/1/2/4, Guidant, Minneapolis, Minnesota, USA and Insync (Marquis) III or Sentry, Medtronic Inc., Minneapolis, Minnesota, USA). Two types of LV leads were used (Easytrak, Guidant, or Attain, Medtronic Inc.).

### Biochemical analysis

Before implantation and after 6 months follow-up, blood samples were obtained, and serum and plasma samples were stored at -80°C. Serum levels of NT-proBNP were determined using an automated immunoassay (Elecsys, Roche). The reference range is 0-400 pg/mL, intra-assay variability was 1.8% at high concentrations of NT-proBNP (800 pg/mL), and 2.7% at low concentrations (2.1 pg/mL). Serum levels of TNC were assayed with the human TNC high molecular weight variants assay kit (ImmunoBiological Laboratories, Hamburg, Germany). The upper limit of normal value is 30.9 ± 8.8 ng/mL, and intra- and inter-assay variations were 6% and 5%, respectively. Activities of MMP-2 and MMP-9 in plasma were assayed by zymography. Plasma samples were applied to polyacrylamide gels containing gelatin. After separation of proteins by electrophoresis, the gel was incubated overnight at 37°C in the presence of 5 mmol/L $CaCl_2$, and 10 µmol/L $ZnCl_2$ and subsequently stained with 0.1% Amido Black[14]. Gelatinase activity was quantified by measuring the extent of gelatin digestion using a scanning densitometer (LKB 2220) after Amido Black staining[14]. The 92 kDa and 72 kDa bands represent the MMP-9 and MMP-2 activities, respectively.

### Statistical analysis

Data were expressed as mean ± SD and compared with the two-tailed Student's t-test for paired and unpaired data when appropriate. Categorical variables were compared using the chi-square test with Yates' correction. A p-value $<0.05$ was considered statistically significant.

## Results

A total of 64 patients were included in the study (52 men and 12 women) with a mean age of 64 ± 10 years. The baseline characteristics of the patients are summarized in Table 1.

At 6 months follow-up, the patient group demonstrated a significant improvement in clinical parameters, as evidenced by an improvement in NYHA class (from 3.1±0.2 to 2.2±0.7, $p<0.001$) and an improvement in 6-minute walking distance from 330±114 m to 406±117 m ($p<0.001$). In addition, echocardiography revealed a significant improvement

in LVEF from 25±8% to 34±10% (p<0.001) and a significant reduction in LV volumes (LVEDV from 228±78 mL to 188±72 mL and LVESV 172±69 mL to 128±62 mL, both p<0.001).

**Table 1**. Baseline characteristics of the study population (n=64).

| | | |
|---|---|---|
| Age (yrs) | | 64 ±10 |
| Gender (M/F) | | 52/12 |
| Etiology | Ischemic | 45 (70%) |
| | Non-ischemic | 19 (30%) |
| QRS duration (ms) | | 162 ± 24 |
| NYHA functional class | | |
| | III | 60 (94%) |
| | IV | 4 (6%) |
| 6-MWT (m) | | 330 ± 114 |
| LVEF (%) | | 25 ± 8 |
| LVEDV (mL) | | 228 ± 78 |
| LVESV (mLl) | | 172 ± 69 |

Abbreviations; 6-MWT, 6-minute walking distance; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; NYHA, New York Heart Association.

**Effects of CRT on biochemical markers**

At 6 months follow-up, the patient group demonstrated a significant reduction in NT-proBNP serum levels compared to baseline (from 1952±1719 pg/mL to 1269±1375 pg/mL, p<0.01). In addition, TNC serum levels decreased from 57±37 ng/mL to 47±2 ng/mL (p<0.001) and MMP-9 plasma levels decreased from 54±27 AU to 46±27 AU (p<0.01). No significant differences were observed for plasma levels of MMP-2 (105±36 AU versus 106±32 AU, n.s.).

**Responders versus non-responders**

At 6 months after CRT implantation, 46 patients (72%) showed a reduction >10% in LVESV and were classified as responders to CRT, whereas 18 patients (28%) with a reduction ≤10% or an increase in LVESV at 6 months follow-up were classified as non-responders. In Table 2, characteristics between responders and non-responders to CRT are compared at baseline and at 6 months follow-up. No significant differences were observed in baseline patient characteristics, except for patient age.

At 6 months follow-up, the responders showed an improvement in NYHA class from 3.1±0.3 to 2.0±0.7 ($p<0.001$). This was associated with an improvement in 6-minute walking distance (Table 2). By definition, responders showed a reduction in LVESV, as well as a reduction in LV end-diastolic volume, reflecting reverse LV remodeling, and an increase in LVEF. Non-responders demonstrated a minimal improvement in NYHA class, without improvement in 6-minute walking distance. Echocardiography revealed even an increase in LV volumes, indicating progression of LV remodeling rather than reverse LV remodeling, without a change in LVEF.

**Biochemical markers in responders versus non-responders**

At baseline, no significant differences in any of the biochemical markers were observed between responders and non-responders (Fig. 1). At 6 months follow-up, the responders to CRT exhibited a significant reduction in NT-proBNP serum levels (Fig. 1a). Also, TNC serum levels and plasma levels of MMP-9 showed a significant reduction at 6 months follow-up (Fig. 1b, 1d). Plasma MMP-2 levels did not decrease in the responders (Fig. 1c). In non-responders, none of these markers showed a reduction at 6 months follow-up (Fig. 1a-d).

**Table 2.** Clinical and echocardiographic variables at baseline and after 6 months of CRT, in responders and non-responders.

| | **Responders (n=46)** | **Non-responders (n=18)** | **p-value** |
|---|---|---|---|
| Age (yrs) | 66 ± 10 | 60 ± 11 | <0.05 |
| Gender (M/F) | 38/8 | 14/4 | NS |
| Etiology (ischemic/non-ischemic) | 30/16 | 15/3 | NS |
| QRS duration (ms) | 165 ± 23 | 153 ± 25 | NS |
| NYHA class | | | |
| baseline | 3.1 ± 0.3 | 3.1 ± 0.2 | NS |
| follow-up | 2.0 ± 0.7* | 2.5 ± 0.7* | <0.05 |
| 6-MWT (m) | | | |
| baseline | 333 ± 114 | 321 ± 116 | NS |
| follow-up | 427 ± 115* | 360 ± 109 | <0.05 |
| LVEF (%) | | | |
| baseline | 24 ± 8 | 28 ± 8 | NS |
| follow-up | 36 ± 9* | 27± 8 | <0.05 |
| LVEDV (mL) | | | |
| baseline | 236 ± 72 | 206 ± 91 | NS |
| follow-up | 178 ± 62* | 214 ± 91* | NS |
| LVESV (mL) | | | |
| baseline | 181 ± 66 | 150 ± 76 | NS |
| follow-up | 116 ± 53 | 157 ± 64 | <0.05 |

Abbreviations; 6-MWT, 6-minute walking distance; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; NYHA, New York Heart Association.

* $p<0.05$ baseline versus 6 months follow-up (paired t-test).

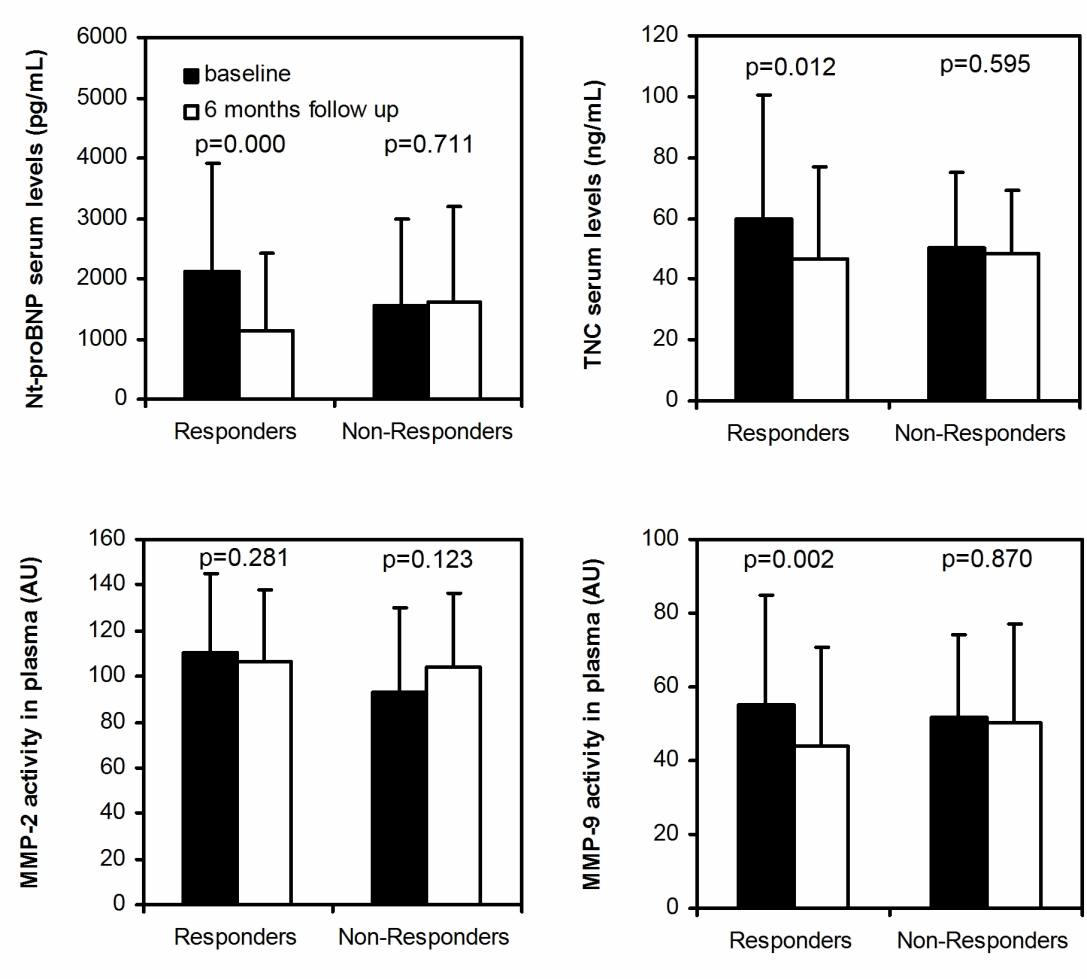


**Figure 1.** Serum or plasma levels at baseline (■) and after 6 months follow-up (□) in responders (n=46) and non-responders (n=18) to CRT. Panel a: amino-terminal propeptide of brain natriuretic peptide (NT-pro BNP), Panel b: tenascin-C (TNC), Panel c: matrix metalloproteinase-2 (MMP-2), Panel d: matrix metalloproteinase-9 (MMP-9).

# Discussion

The main finding of this study is that response to CRT in heart failure patients is associated with a decrease in circulating levels of NT-proBNP, TNC and MMP-9. This finding suggests an important role of ECM modulation in the process of reverse ventricular remodeling in patients responding to CRT.

CRT in patients with moderate-to-severe heart failure has been demonstrated to result in a sustained improvement of clinical symptoms and LV systolic function[2]. Similar benefits were noted in the current study. Clinical parameters and LVEF had improved

significantly and LV volumes were reduced, indicating reverse LV remodeling. Nevertheless, the underlying mechanisms of reverse LV remodeling following CRT are not completely understood. Adverse LV remodeling during the progression of heart failure involves multi-step reactions and is accompanied by changes in structure and composition of the myocardial ECM. Several proteins have been demonstrated to be involved in ECM remodeling, including MMPs[15] and TNC[16]. As ECM remodeling may play an important role in reverse LV remodeling following CRT, we studied several proteins known to be involved to ECM metabolism. Specifically we studied whether TNC, MMP-2, MMP-9, and NT-proBNP in patients with heart failure undergoing CRT implantation are indicative of reverse remodeling.

TNC is a large ECM protein that is primarily expressed in the fetal myocardium but is not detected in the adult heart[17]. However, it is re-expressed in the adult heart under various pathological conditions associated with extensive tissue remodeling, including acute myocardial infarction[18], myocarditis[19], and dilated cardiomyopathy[20]. TNC contributes to adverse LV remodeling by weakening the binding between cardiomyocytes and the ECM, leading to cardiomyocyte slippage, LV dilatation, and a reduction in contractile function[16]. Elevated serum levels of TNC may reflect TNC expression in myocardial tissue and a recent study by Sato et al.[10] reported that serum TNC may be a novel predictor of adverse LV remodeling and prognosis after acute myocardial infarction.

By definition, responders showed a reduction in LVESV, as well as a reduction in LV end-diastolic volume, reflecting reverse LV remodeling. In the current study we demonstrated that responders also showed a reduction in TNC serum levels, whereas TNC serum levels in non-responders remained unchanged. These results suggest that reverse LV remodeling in response to CRT may be the result of decreased levels of myocardial TNC, leading to a reduction in cardiomyocyte slippage, and an improved cardiac function.

In addition to its de-adhesive properties, TNC also interacts with other remodeling-related proteins and several studies demonstrated co-expression of TNC with MMPs in areas of active tissue remodeling[21]. MMPs, in particular MMP-2 and MMP-9, play an important role in ECM degradation and structural changes associated with ventricular remodeling[15]. MMPs are synthesized as a latent proform that can be converted to an

active MMP form by enzymatic cleavage of the propeptide domain by plasmin or other MMPs in response to mechanical stress[22]. The actions of MMPs are dependent on the balance between the enzymes and their inhibitors[23], indicating a complex regulation. Activation of MMPs leads to progressive degradation of connective tissue, cardiomyocyte slippage, ventricular wall thinning, ventricular dilatation, and finally results in an impaired cardiac function[15]. Plasma levels of MMP2 and MMP9 have been demonstrated to correlate positively with LV volumes and negatively with LV ejection fraction[24;25], indicating that MMP plasma levels may reflect the MMP activity within the myocardium. Both MMP-2 and MMP-9 plasma levels are increased in patients with congestive heart failure and are reported to be biochemical markers of adverse ventricular remodeling[8;9]. Although MMP plasma levels have been demonstrated to correlate with ventricular function, evaluation of circulating MMP levels as a marker of ventricular remodeling has some limitations due to the complex regulation of MMP expression, activation and inhibition within the myocardium. Pharmacologic MMP inhibition has been used in animal models of LV dysfunction and leads to lesser LV dilatation[26].

In the current study we demonstrated that plasma MMP-9 activity in responders was reduced but remained unchanged in non-responders. These results suggest that reverse ventricular remodeling may be associated with a reduction of MMP-9 activity in the ECM and may lead to reduced ECM degradation, attenuation of LV dilatation, and improved cardiac function. These results are consistent with a previous study by Li et al.[27],which reported downregulation of MMP-9 gene expression in patients with dilated cardiomyopathy following implantation of a LV assist device.

In contrast, plasma MMP-2 activity in both responders and non-responders remained unchanged. This suggests a differential activation of MMP-9 and MMP-2 in ventricular remodeling. Indeed, Nishikawa et al.[28] reported that MMP-9 rather than MMP-2 plays an important role in LV dilatation, which may explain our finding of decreased MMP-9 activity in relation to reverse ventricular remodeling in response to CRT. Previously, it was shown that TNC activates MMPs, including MMP-9 via TGF-β[29]. In the present study we found that TNC levels are reduced in responders to CRT, which may explain the reduced MMP-9 activity in plasma of these patients.

Brain natriuretic peptide (BNP) and the N-terminal fragment of its prohormone (NT-proBNP) are released from cardiomyocytes in response to increased ventricular wall stress, the major forces driving LV remodeling[30]. In patients with congestive heart failure both BNP[31] and NT-proBNP[11] were found to be predictors for adverse ventricular remodeling. The precise role of BNP in regulation of ECM metabolism remains unclear but it was found that BNP can increase MMP production, in particular MMP-9[32]. Reverse ventricular remodeling after implantation of a LV assist device in patients with heart failure was associated with decreased myocardial BNP gene expression and decreased BNP plasma concentrations[33]. These findings corroborate the finding in the current study that NT-proBNP levels were reduced in serum of patients with reverse LV remodeling in response to CRT, but remained unchanged in the non-responders without reverse remodeling. This indicates that NT-proBNP is a relevant biochemical marker of reverse ventricular remodeling and can be used to evaluate the response to CRT.

**Conclusions**

CRT improves clinical symptoms and LV function in the majority of heart failure patients. Reverse ventricular remodeling after CRT was associated with decreased circulating levels of TNC, NT-proBNP and MMP-9. Therefore, these biochemical markers of reverse ventricular remodeling may be used to evaluate the response to CRT.

**Limitations of the study**

In this study, the number of patients was relatively small and a wider population is needed to confirm our findings. Although MMP plasma levels have been demonstrated to correlate with LV volumes and LV ejection fraction suggesting that MMP plasma levels may reflect the myocardial MMP activity, evaluation of circulating MMP levels as a marker of ventricular remodeling has some limitations.

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