Serum Markers of Collagen Turnover Predict Future Shocks in Implantable Cardioverter-Defibrillator Recipients With Dilated Cardiomyopathy on Optimal Treatment

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Objectives
We investigated prospectively whether serum markers of collagen turnover could be used as predictors for the occurrence of malignant ventricular arrhythmias in patients with nonischemic dilated cardiomyopathy (NIDC) who had received an implantable cardioverter-defibrillator (ICD) for primary prevention.

Background
Extracellular matrix alterations in NIDC might provide electrical heterogeneity, thus potentially contributing to the occurrence of ventricular arrhythmia and subsequent sudden cardiac death (SCD).

Methods
Serum C-terminal propeptide of collagen type-I, C-terminal telopeptide of collagen type-I, matrix metalloproteinase (MMP)-1, and tissue inhibitor of MMP-1 were measured as markers of collagen synthesis and degradation in 70 patients with mild to moderate symptomatic heart failure due to NIDC with left ventricular ejection fraction <35%, who received an ICD for primary prevention of SCD. Patients were evaluated for any appropriate ICD-delivered therapy, whether shock or antitachycardia pacing, during a 1-year follow-up period.

Results
Appropriate device therapies were delivered in 14 of the 70 patients during the follow-up period, with antitachycardia pacing in 2, antitachycardia pacing with shocks in 4, and shocks in 8. Pre-implantation serum concentrations of C-terminal telopeptide of collagen type-I levels were significantly higher in patients who had appropriate ICD-delivered therapy than in those who did not have any therapy (0.46 ± 0.19 ng/ml vs. 0.19 ± 0.07 ng/ml, p < 0.001, respectively). The same was true for baseline MMP-1 and tissue inhibitor of MMP-1 (27.7 ± 1.6 ng/ml vs. 24.1 ± 2.5 ng/ml, p < 0.001, and 89 ± 14 ng/ml vs. 58 ± 18 ng/ml, p = 0.008, respectively).

Conclusions
If the maximum benefit is to be achieved from ICD therapy in NIDC patients for the primary prevention of SCD, a more precise risk stratification is required. As extracellular matrix alterations affect the arrhythmogenic substrate in NIDC, we observed that serum markers of collagen turnover could predict arrhythmic events in ICD recipients. (J Am Coll Cardiol 2010;55:2753–9) © 2010 by the American College of Cardiology Foundation

During the last few years, several trials have been published that showed a survival benefit for implantable cardioverter-defibrillator (ICD) therapy over antiarrhythmic drugs alone, used for the primary prevention of sudden cardiac death (SCD) in patients with nonischemic dilated cardiomyopathy (NIDC) (1–4). Therefore, according to these trials ICD therapy should be considered in these patients. In recent practice guidelines, ICD insertion in patients with NIDC is based only on the degree of left ventricular dysfunction, as estimated by the ejection fraction, and on the functional stage of congestive heart failure quantified by New York Heart Association (NYHA) functional class (5). However, the discussion continues, largely because it is still not clear how the selection of patients could be optimized. To provide the maximum benefit from ICD therapy, individual prediction is required to discriminate between patients who are at increased risk for SCD and those with a negligible risk, who would be exposed to potential adverse effects without benefit. With this in mind, it would be highly
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Methods

Patient selection. The ethics committee of our institution approved the study. This investigation conforms to the principles outlined in the Declaration of Helsinki. Signed informed written consent was obtained from all subjects before their participation in the study.

The study population consisted of 70 patients with NIDC, who had either attended or been referred to our clinic, the only tertiary cardiology care center on the island, for evaluation and ICD insertion. All patients had NYHA functional class II to III heart failure, a left ventricular ejection fraction (LVEF) <35%, and might better identify patients at risk as early as possible.

It is known that the remodeling process in NIDC is characterized by changes in extracellular matrix (ECM), including fibrosis formation and collagen degradation (6–8). The ECM alterations might provide electrical heterogeneity, which is an appropriate substrate for arrhythmogenesis, thus potentially contributing to the occurrence of ventricular arrhythmia and subsequent SCD (9). Experimental and clinical data suggest that biochemical markers of collagen turnover correlate significantly with fibrosis in endomyocardial biopsies in hypertensive patients (10). Serological markers of these processes seem to be important targets of therapy and have been used to provide information about prognosis in patients with symptomatic heart failure (11) and idiopathic or ischemic dilated cardiomyopathies (12) and after acute myocardial infarction (13).

In this study we planned to investigate prospectively whether serum markers of collagen turnover could be used to provide information about prognosis in patients with symptomatic heart failure (11) and idiopathic or ischemic dilated cardiomyopathies (12) and after acute myocardial infarction (13).

Desirable to discover prognostic clinical or laboratory indexes that reflect the arrhythmia substrate and might better identify patients at risk as early as possible.

The patients were clinically stabilized by treatment with a combination of aldosterone antagonists, angiotensin-converting enzyme inhibitors, or angiotensin II receptor blockers and carvedilol.

Study design. BIOCHEMICAL MEASUREMENTS OF MARKERS OF COLLAGEN TURNOVER. Because collagen type-I is the major collagenous product of cardiac fibroblasts (14), we used as markers of collagen turnover serum peptides derived from the tissue synthesis (C-terminal propeptide of collagen type-I [CICP]) and degradation (C-terminal telopeptide of collagen type-I [CITP]) of collagen type-I (10). Enzymes that control collagen type-I turnover, specifically matrix metalloproteinase (MMP)-1 and tissue inhibitor of matrix metalloproteinases (TIMP)-1, were also measured (15).

Blood samples were obtained at the beginning of the study, before placement of the ICD, by direct puncture of an antecubital vein after 30 min of supine rest, and were immediately placed on ice and centrifuged within 1 h. Specimens were stored at −80°C until analysis.

The CICP levels were determined by a sandwich enzyme immunoassay with a commercially available kit (enzyme immunoassay, Metra CICP, Quidel, San Diego, California), whereas CITP was measured with the Elecsys beta-CrossLaps/serum assay (Roche Diagnostics, Mannheim, Germany). Serum MMP-1 and TIMP-1 levels were assayed by enzyme-linked immunoabsorbent assay with commercially available kits (Human Biotrack enzyme-linked immunoabsorbent assay system, Amersham Biosciences, Piscataway, New Jersey). Measurements were performed in duplicate by personnel blinded to the patients’ clinical details. The intra- and inter-assay coefficients of variation of all assays were <8% and <10%, respectively, in our laboratory.

DEVICE FOLLOW-UP AND DATA COLLECTION. After implantation, a complete evaluation of device function was performed, and the tachycardia therapies and detection rates were programmed. A VT detection zone with antitachycardia pacing therapy was programmed in all patients, followed by shocks if necessary. Above 200 beats/min, only shock therapies were programmed. All patients were followed-up in the implantable device clinic of our department every 3 months or sooner in cases of device discharges, thus ensuring comprehensive data collection.
Data concerning arrhythmias and device therapy were obtained and stored at the time of device interrogation on each follow-up visit. The incidence and type of arrhythmias and the incidence of appropriate and inappropriate defibrillator therapies were determined by reviewing stored electrograms. Events were classified by an agreement of 2 reviewing electrophysiologists blinded to the study. Ventricular tachyarrhythmias were categorized as VT or ventricular fibrillation (VF) on the basis of rate and morphology and by the type of device therapy (antitachycardia pacing or shock) that terminated the tachyarrhythmia. Appropriate antitachycardia pacing or shock was defined as any ICD therapy that occurred in response to VT or VF, whereas such an event was classified as inappropriate when it was triggered by supraventricular tachycardias or T-wave oversensing or when it was secondary to electrode dysfunction.

On each visit, an appropriate clinical and laboratory evaluation, which included cardiac troponins, plasma electrolytes, and thyroid hormones to exclude factors potentially triggering arrhythmias, was carried out. Medication use was also recorded.

The end point of the study was the occurrence of any appropriate ICD delivered therapy, whether shock or antitachycardia pacing, for malignant ventricular arrhythmias during a 1-year follow-up period.

**Statistical analysis.** Summary descriptive data are presented as mean ± SD for continuous variables and counts (proportions) for categorical variables. Patients were classified into 2 groups: group I included patients who experienced at least 1 VT during the study period, and group II included patients without VT during the study period.

Categorical variables were compared between groups with a Pearson chi-square test or a Fisher exact test (when chi-square was not applicable). Continuous variables were compared with a t test or a Mann-Whitney U test, as appropriate. Receiver-operating characteristic curves showing the diagnostic performance of serum markers were constructed. The association between continuous parameters was assessed with Pearson’s correlation coefficient. A stepwise logistic regression model was employed to explore the potential impact of significant predictors for shocks. Values of p < 0.05 were considered statistically significant. The software SPSS version 17 (SPSS Inc., Chicago, Illinois) was used for our analysis.

**Results**

**Baseline characteristics.** The patient population included 12 women and 58 men, ranging in age from 41 to 77 years. Thirty-seven patients were classified as NYHA functional class II, and 33 were classified as NYHA functional class III. The mean LVEF was 25.1 ± 7%, and the mean LVEDD was 60.4 ± 6 mm. Patients were receiving optimal medical therapy for NICM, which included carvedilol, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, or aldosterone antagonists, unless there was any contra-indication or patient intolerance. Digoxin and diuretics were used when necessary to manage clinical symptoms.

**Clinical outcome and collagen turnover levels.** All patients underwent their routine follow-up examination, none withdrew, and no patient died during the study period. Appropriate device therapies were delivered in 14 of the 70 patients during the 1-year follow-up period, with antitachycardia pacing in 2, antitachycardia pacing with shocks in 4, and shocks in 8.

The initial arrhythmia triggering defibrillator activity was monomorphic VT in 9 patients and VF in 5 patients. Among these 14 patients, 8 patients had 1 tachyarrhythmic event, and the remaining 6 had 2 or more episodes.

Apart from the 14 patients who experienced appropriate device therapies, 3 patients experienced inappropriate ICD discharge due to atrial fibrillation.

**Table 1** presents the baseline characteristics of patients with and without appropriate ICD-delivered therapy. Age, sex, NYHA functional class, LVEF, LVEDD, and medications did not differ between these 2 groups. No difference was observed between the 2 groups with regard to the presence of diabetes mellitus or atrial fibrillation and kidney disease or the dosage of carvedilol. There was no difference between the groups with regard to the selection criterion for device implantation (i.e., if patients had nonsustained VT on Holter monitoring or VT induction during programmed ventricular stimulation) (p = 0.99). For CICP levels the difference between the 2 groups was not significant (56.7 ± 14 ng/ml vs. 56.2 ± 12 ng/ml, p = 0.90).

Pre-implantation serum concentrations of CITP levels were significantly higher in patients who had appropriate ICD-delivered therapy than in those who did not have any therapy (0.46 ± 0.19 ng/ml vs. 0.19 ± 0.07 ng/ml, p < 0.001, respectively) (Fig. 1). The same was true for baseline MMP-1 and TIMP-1 (27.7 ± 1.6 ng/ml vs. 24.1 ± 2.5 ng/ml).

| Table 1 Baseline Characteristics of Patients Who Did and Did Not Receive Therapy From an ICD |
|-----------------|-----------------|-----------------|
|                  | ICD Therapy (n = 14) | No ICD Therapy (n = 56) | P Value |
| Age (yrs)       | 65 ± 21          | 65 ± 25          | 0.99    |
| Men             | 12 (86%)         | 45 (80%)         | 0.95    |
| NYHA functional class II | 8 (57%)     | 29 (52%)         | 0.77    |
| NYHA functional class III | 6 (43%) | 27 (48%)         | 0.77    |
| LVEF (%)        | 23 ± 4           | 25 ± 7           | 0.27    |
| LVEDD (mm)      | 61 ± 5           | 60 ± 6           | 0.30    |
| Atrial fibrillation | 2 (14%)       | 12 (16%)         | 0.99    |
| Diabetes mellitus | 4 (28%)      | 13 (23%)         | 0.73    |
| Kidney disease  | 1 (7%)           | 13 (9%)          | 0.99    |
| ACE inhibitors or ATII blockers | 13 (93%)  | 54 (96%)         | 0.49    |
| Carvedilol | 14 (100%) | 55 (98%) | 0.99 |
| Dosage of carvedilol (mg/day) | 24.5 ± 18 | 23.6 ± 16 | 0.85 |
| Aldosterone antagonists | 11 (78%) | 43 (76%) | 0.99 |

Data are expressed as mean ± SD or n (%) unless otherwise noted. None of the differences between groups were statistically significant.

ACE = angiotensin converting enzyme; ATII = angiotensin II receptor; ICD = implantable cardioverter-defibrillator; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.
ng/ml, p < 0.001, and 89 ± 14 ng/ml vs. 58 ± 18 ng/ml, p = 0.008, respectively) (Figs. 2 and 3). To compare the aforementioned 3 markers in terms of their sensitivity and specificity we constructed receiver-operating characteristic curves. As can be seen in Figure 4 CITP perform better than the other 2 markers. More specifically the area under the curve for CITP, MMP-1, TIMP-1 is 0.95, 0.883, and 0.782, respectively. Moreover a CITP cutoff point of 0.28 ng/ml was found to be 85.7% sensitive and 89.3% specific for the prediction of malignant arrhythmic events. Similarly an MMP-1 cutoff value of 26 ng/ml yields 78.6% sensitivity and 81% specificity. For TIMP-1 a cutoff point of 45 ng/ml had 85.7% sensitivity and 60.7% specificity for the occurrence of ventricular arrhythmias that require appropriate ICD-delivered therapy.
No significant correlation was observed between the aforementioned serum markers of collagen turnover and LVEF ($r = -0.185$, $p = 0.124$ for CITP, $r = -0.057$, $p = 0.64$ for MMP-1, and $r = -0.179$, $p = 0.138$ for TIMP-1).

Additionally, despite the limited patient number, for exploratory reasons only, we performed a stepwise logistic regression analysis, evaluating the 3 serum markers of collagen turnover that were univariately significant. The results indicate that CITP (beta = 31.2, $p = 0.001$) and TIMP-1 (beta = 0.07, $p = 0.023$) carry independent prognostic information that should be verified in a larger sample.

Discussion

Because the survival benefit of ICD in NIDC patients with poor left ventricular function has become evident after the publication of SCD-HeFT (Sudden Cardiac Death in Heart Failure Trial) (4), current guidelines recommend their implantation in this vulnerable population (5). However, the early and reliable identification of patients at higher risk of SCD who could benefit from an ICD or, perhaps more importantly, those who are unlikely to benefit remains an unresolved issue.

It is a fact that, even in high-risk population groups, not all patients have the same risk of malignant arrhythmias. A number of specific techniques that detect slowed conduction, heterogeneities in ventricular repolarization, imbalance in autonomic tone, extent of myocardial damage, and ventricular ectopy have been evaluated (16).

Although many studies have explored the value of these techniques, the precise relationship between the presence of these abnormalities, some of which are persistently present, and the unpredictable occurrence of VT/VF has not been elucidated (17-20). Even abnormalities in combinations of these techniques might fail to detect the precise pathophysiological abnormalities that precipitate VT or VF. Therefore the limitations of these techniques might be due in part to our inadequate understanding of the milieu responsible for initiating clinical episodes of VT or VF.

Newer approaches that encompass a more general evaluation of “vulnerability” to SCD, including serum markers, genetic profiling, and new imaging approaches, are necessary.

The present study identifies some serum markers of collagen turnover as predictors of major arrhythmic events in mild to moderate symptomatic heart failure patients due to NIDC with LVEF <35%.

The ECM of the myocardium, which is mainly composed of type I collagen fibers, plays an important role in the remodeling process during the development of NIDC (21,22). In the normal heart, there is a balance between collagen synthesis by myofibroblasts and its degradation by proteolytic enzymes (MMPs) (23). In NIDC, the fibrotic process is characterized initially by degradation of properly cross-linked type I collagen, followed by increased reparative fibrosis (24). This means that altered ratios of collagen subtypes, pathologically produced, replace myocyte cells that are dead as a result of apoptosis or necrosis. The focal accumulation of functionally unfavorable collagen and the degradation of relevant architecture of perimysial and endomysial collagen create a barrier to impulse propagation and interfere with conduction by impairing intermyocyte coupling, creating an ideal arrhythmogenic substrate (25).

A number of experimental and clinical data suggest that biochemical markers of collagen turnover correlate significantly with fibrosis in endomyocardial biopsies; thus, serum concentrations of procollagen propeptides can be used as markers of myocardial fibrosis formation or collagen degradation (26-28).

In our patients, ventricular arrhythmias were associated with higher serum levels of CITP, a degradation marker of type I collagen, and this collagenolytic activity was confirmed by the increased concentrations of MMP-1 in the same patients. Furthermore, because all of our patients had the diagnosis of NIDC for a long period, which extended beyond the early phase of the disease, we might suppose that the process of replacement fibrosis had started. This might explain the significantly increased concentration of TIMP-1, the inhibitor of collagenolysis, in patients more prone to malignant arrhythmias.

To our knowledge, only 1 study by Blangy et al. (29) similarly investigated the relationship of cardiac fibrosis and the incidence of ventricular arrhythmias—though this was in secondary prevention ICD recipients after myocardial infarction—and showed that the combination of decreased pro-collagen type III and increased pro-collagen type I was a significant marker of the risk of VT in the multivariate analysis.

Another interesting finding was that both CITP and TIMP-1 could provide prognostic information above and beyond other conventional risk markers in our sample of NIDC patients, who were typical candidates for ICD insertion in daily clinical practice. Because all of our patients were under the same optimal pharmaceutical treatment and whereas patients with sustained ventricular arrhythmias who received appropriate therapy from their devices presented with no difference in the diameter or function of the left ventricle compared with patients without sustained arrhythmias, we can speculate that the predisposition to ventricular arrhythmias could be attributed to the fibrosis process and not to the presence of any confounding factor. Perhaps surprisingly, spironolactone, which was taken by a relatively high proportion of patients in both groups and is known to affect collagen turnover (11), did not seem to affect the risk of malignant arrhythmias. However, that could be explained by the relatively small number of arrhythmic events and that our patients had mild to moderate symptomatic heart failure, in contrast to the severe heart failure population in RALES (Randomized Aldactone Evaluation Study) (11).

Study limitations. Serum markers of collagen turnover are not heart-specific. In addition, we did not support our findings with cardiac tissue biopsy data or coronary sinus...
sampling. However, we made strenuous efforts to exclude subjects who had conditions associated with fibrosis.

Although the population of our study was relatively homogenous, the relatively low VT occurrence rate would have necessitated a larger sample of patients with a longer follow-up and repeated measurements of serum markers. Our analysis was based on a single determination of each serum marker, which did not allow assessing for the potential changes in these markers over time. It is possible that, with a longer follow-up and a larger number of events, the predictive power of serum markers of collagen turnover for ICD therapy could have been determined with more precision.

Finally, because some medications for the treatment of heart failure, such as aldosterone blockers, might alter ECM turnover (11,30) they might have had some influence on our patients’ outcomes. However, the medications were not interrupted for ethical reasons. In any case, we think that this factor would have had a minimal effect, because all patients were under the same optimal treatment, without any differences between the 2 groups. Another limitation, as implied in the final paragraph of the Results section, is the potential over-fitting of the multivariate logistic regression model.

Given these limitations, this is the first study to estimate the relationship between ICD shocks and serum markers of collagen type-I synthesis and degradation, demonstrating a potential role for these markers in the risk of malignant arrhythmias in patients with NIDC.

Conclusions

If the maximum benefit is to be achieved from ICD therapy in NIDC patients for the primary prevention of SCD, more precise risk stratification is required. We observed that, because ECM alterations affect the arrhythmogenic substrate in NIDC, serum markers of collagen turnover could predict arrhythmic events in ICD recipients. Although these results are encouraging, further large prospective studies are necessary to determine the positive and negative predictive accuracy of these biomarkers and to investigate their predictive ability when compared with previously better-defined risk factors.

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