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Multimarker Approach to Risk Stratification Among Patients with Advanced Chronic Heart Failure

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Summary

Background: Cardiac troponin I (cTnI), N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity C-reactive protein (hsCRP) each predict adverse cardiac events in chronic heart failure (CHF). However, little is known about the utility of these novel biomarkers of CHF in combination.

Hypothesis: We hypothesized that simultaneous assessment of the three biomarkers would enable clinicians to stratify risk more effectively among patients with advanced CHF.

Methods: Measurements of the biomarkers were performed on 152 patients with symptomatic advanced CHF. Major adverse cardiac events during a median follow-up period of 186 days were determined.

Results: Univariate and multivariate analysis revealed that elevations of each biomarker were significant predictors of clinical outcome independently of clinical variables. When patients were categorized on the basis of the number of elevated biomarkers, patients with one, two and three elevated biomarkers respectively had a 2.7-($p = 0.125$), 8.6- ($p < 0.0001$) and 23.4-($p < 0.0001$) fold increase in the risk of adverse events.

Conclusions: Simultaneous measurement of cTnI, hs-CRP, and NT-proBNP could provide complementary information and a simple multimarker strategy that categorizes the patients with advanced CHF based on the number of elevated biomarkers, allowing rapid risk stratification in these patients.

Key words: congestive heart failure, biomarkers, prognosis

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Introduction

To properly target heart failure populations for heart transplantation or newer therapies such as biventricular pacing or insertion of mechanical support devices, the identification of patients with chronic heart failure (CHF) at higher risk of death or recurrent hospitalization is of paramount importance.[1]

Several new biomarkers have emerged as strong predictors of risk among patients presenting with cardiovascular disease, including CHF. Among them, cardiac troponin I (cTnI) and T,[2] B-type natriuretic peptide (BNP),[3] N-terminal pro-B-type natriuretic peptide (NT-proBNP)[4] and high-sensitivity C-reactive protein (hsCRP)[5,6] are easy to measure and such measurement is now routinely available to clinicians. Sabatine *et al*. have demonstrated that simultaneous assessment of cTnI, CRP, and BNP could enable clinicians to stratify risk more effectively among patients with acute coronary syndrome.[7] Although a multimarker approach to risk stratification has been advocated for some time by several experts,[7,8] it has not, to the best of our knowledge, been reported before for patients with CHF.

Since the aforementioned biomarkers may also assess different pathophysiological mechanisms in CHF—elevations in cardiac troponins may indicate ongoing myocardial cell injury associated with the progression of CHF,

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BNP and NT-proBNP are elevated in response to left ventricular overload, and hsCRP is a marker of systemic inflammation—we hypothesized that simultaneous assessment of cTnI, NT-proBNP and hsCRP would provide complementary information and enable clinicians to stratify risk more effectively among patients with advanced CHF. The aim of this study was to test this hypothesis using a similar approach to that proposed by Sabatine *et al*.[7]

Methods

Patient Population

We studied 152 consecutive patients 42–70 years old (mean 56 ± 14 years; 117 men, 35 women) referred for clinical heart failure management and/or transplantation evaluation. All of them had New York Heart Association (NYHA) functional classification of II to ambulatory class IV despite optimal medical treatment and proper diagnosis for at least 2 months and had a left ventricular ejection fraction (LVEF) of <35% by echocardiography. Patients were not allowed to participate if they had any of the following occurring within two weeks before entry into the study: change in NYHA functional class, change in heart failure medications, or administration of any intravenous medication for heart failure. The study protocol was approved by the institutional committee on human research, and all participants gave informed consent.

Exclusion criteria included chronic obstructive pulmonary disease, primary valvular heart disease, infection or an inflammatory illness such as sepsis, malignancy, arthritis or connective tissue disease, pregnancy, and severe liver disease as defined by hepatic enzymes >2 times the upper limit of normal.

The etiology of CHF was determined as ischemic when coronary angiography revealed >70% luminal diameter narrowing in at least one major epicardial coronary artery. In those patients with CHF without coronary artery disease for whom the endomyocardial biopsy revealed findings compatible with dilated cardiomyopathy, the cause of CHF was determined as dilated cardiomyopathy.

Blood Sampling and Measurement of Circulating Levels of Biomarkers

Blood samples were collected from an indwelling catheter into vacuum tubes at bedside (peripheral vein) with patients in the supine position for at least 30 minutes. The serum was separated by centrifugation immediately and then frozen to −20°C and was stored at that temperature until analysis.

The cardiac troponin I were measured with an Abbott Axsym system (Abbott Laboratories, Abbott Park, IL, USA). The NT-proBNP was determined with Roche ElecsysNT-proBNP (Roche Diagnostics GmbH), a quantitative electrochemiluminescence immunoassay, using an Elecsys 2010 immunoassay analyzer (Roche Diagnostics GmbH). Samples were also measured by immunoassay for hsCRP by the use of an autoanalyzer (IMMAGE Immunochemistry Systems, Beckman Coulter, Inc. California, USA). The intraassay and interassay coefficients for each factor were about 5 and 10%, respectively, in our laboratory.

Clinical Follow-up

All patients were followed through regular outpatient visits. Clinical information regarding major adverse cardiac events (cardiac death, requirement for heart transplantation or hospitalization with a primary diagnosis of worsening heart failure) during a median follow-up period of 186 days was provided by the cardiologists in charge without knowledge of the biomarker levels.

Data Analysis

All values except for the biomarker levels are expressed as mean ± SD. The biomarker levels in these patients did not follow a normal distribution and so were expressed by medians (25th to 75th percentiles).

The CHF patients were divided into those who had major adverse cardiac events during follow-up and those who were event-free. Univariate comparisons of clinical characteristics and levels of biomarkers between these two groups were made with appropriate tests. Kaplan–Meier analyses of cumulative event-free rates were done with the CHF patients being stratified into two groups on the basis of median LVEF and median levels of biomarkers. The differences between event-free curves were tested by a log rank test. Cox proportional hazards analysis was performed to determine the significance of age, gender, LVEF, presence of ischemic heart disease, systolic blood pressure, serum sodium level, creatinine clearance and circulating levels of cTnI, NT-proBNP and hsCRP as independent predictors of CHF.

A p-value<0.05 was considered statistically significant.

Results

Patient Characteristics

The baseline characteristics of the 152 patients are shown in Table 1. On blood sampling, all patients were receiving continuous therapy for CHF. The causes of CHF were ischemic heart disease in 51 (34%) patients and dilated cardiomyopathy in 101 (66%). The mean LVEF was 26 ± 5%; 41 (27%) patients were in NYHA class II, 63 (41%) were in class III, and 48 (32%) were in ambulatory class IV.

TABLE 1 Baseline clinical characteristics of the 152 study patients

Age (years)	56 ± 14
Left ventricular ejection fraction (%)	26 ± 5
Male, n (%)	117 (77)
Causes of heart failure	
Ischemic heart disease (%)	51 (34)
Nonischemic heart disease (%)	101 (66)
New York Heart Association functional class	
II (%)	41 (27)
III (%)	63 (41)
IV (%)	48 (32)
Medications	
Diuretics (%)	144 (95)
ACEI/ARB (%)	135 (89)
Beta-blockers (%)	93 (61)
Digitalis (%)	74 (49)
Vasodilators (%)	70 (46)

ACEI/ARB = angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers.

Circulating Levels of the Three Biomarkers and Their Interrelations with One Another in Patients with CHF

Not all of the patients with CHF had elevated biomarker levels, and there is clearly a wide range of elevation of the biomarkers in the study population. The correlation coefficients between circulating levels of cTnI and those of NT-proBNP and of hsCRP were 0.444 ($p < 0.0001$) and 0.064 ($p = 0.434$) respectively. The correlation coefficient between circulating levels of NT-proBNP and of hsCRP was 0.081 ($p = 0.320$).

Prognosis

The median follow-up period was 186 days (57–394 days, 25th–75th percentiles), and there was a 41% (63 of 152) overall event rate.

The 152 CHF patients were divided into those who had major adverse cardiac events during follow-up and those who were event-free (Table 2). There were more patients with CHF of coronary disease origin than of nonischemic origin ($p = 0.0103$) in the group with major adverse cardiac events as compared to the event-free group. The use of heart failure medications was similar in both groups. The systolic blood pressure, serum sodium level, and creatinine clearance were significantly lower and the concentrations of all three biomarkers were significantly higher in the group with major adverse cardiac events than in the event-free group.

This is further illustrated by a Kaplan–Meier analysis of event-free survival of the two groups on the basis of median LVEF and median levels of the three biomarkers in Figure 1. The differences in event-free survival curves between two groups were all significant for the three biomarkers (cTnI, $p < 0.0001$; NT-proBNP, $p < 0.0001$; and hsCRP, $p = 0.0002$).

According to the Cox proportional hazards analysis, the three biomarkers remained independent significant predictors of outcome (hazard ratio [HR] for cTnI 2.658, 95% CI 1.431–4.937, $p = 0.002$; HR for NT-proBNP 2.560, 95% CI 1.360–4.821, $p = 0.004$; and HR for hsCRP 2.083, 95% CI 1.197—3.627, p = 0.009) after adjustment of clinical variables that were considered to reflect severity of CHF at baseline and that were associated with adverse events (Table 3).

Kaplan–Meier Analysis of Event-free Survival in Patients with CHF Stratified into Four Groups Based on the Number of Elevated Biomarkers

Since the aim of this study was to test the hypothesis that simultaneous assessment of all three biomarkers would enable clinicians to stratify risk more effectively among patients with advanced CHF, we constructed survival curves after dividing the study participants into four groups. The study patients were categorized on the basis of the number of elevated biomarkers in the Figure 2. Thirty-four (22%) of the 152 patients had elevations in none of the biomarkers, 42 (28%) of them had an elevation in one, 43 (28%) had elevations in two and 33 (22%) had elevations in all three.

Although the difference in event-free survival curves between patients with no elevated biomarkers and those with just one was insignificant ($p = 0.163$), the differences between patients with zero or one elevated biomarker and those with two or three, and between patients with two and those with three elevated biomarkers, were all significant. Patients with one, two and three elevated biomarkers had a 2.7–($p = 0.125$), 8.6–($p < 0.0001$) and 23.4–($p < 0.0001$) fold increase, respectively, in the risk of adverse events.

Discussion

Because no single pathophysiological model can account for the host of clinical expressions of heart failure, a multiaxis framework was proposed in order to appreciate more completely the pathophysiology of CHF.[9] Therefore, a multimarker approach for a better and more complete biochemical characterization of CHF is attractive and is expected to provide more diagnostic and prognostic power as it will combine the intrinsic properties of different proteins.[7,8,10–12] However, most previous studies used one natriuretic peptide in conjunction with one necrosis marker for risk stratification.[10–12] Although Sabatine *et al.* reported that a multimarker approach was feasible and could enable clinicians to better stratify risk among acute coronary syndrome patients,[7] little is known about the utility of three or more biomarkers in combination among patients with CHF.

From a histopathological perspective, necrosis and apoptosis are two fundamental forms of cardiac cell

TABLE 2 Characteristics of patients who had major adverse cardiac events during follow-up vs. those who were event-free

	MACE (–) (n = 89)	MACE (+) (n = 63)	p-value
Age (yrs)	54 ± 15	58 ± 13	0.125
Male, n (%)	71 (80)	46 (73)	0.436
Causes of heart failure			
Ischemic heart disease	22	29	0.010
Nonischemic heart disease	67	34	0.010
Medications, n (%)			
Diuretic (%)	84 (94)	60 (96)	0.892
ACEI/ARB therapy (%)	82 (92)	53 (84)	0.200
Beta-blockers (%)	57 (64)	36 (57)	0.489
Digitalis (%)	40 (45)	34 (54)	0.351
Vasodilator therapy (%)	38 (43)	32 (51)	0.411
Left ventricular ejection fraction (%)	27 ± 5	25 ± 5	0.125
Systolic blood pressure, mmHg	120 ± 18	106 ± 13	0.001
Serum sodium level, mmol/L	139 ± 3	135 ± 4	0.043
Creatinine clearance, mL/min	65 ± 31	47 ± 20	0.010
Cardiac Troponin I (ng/mL)	0.02 (0.00–0.20)	0.20 (0.03–0.68)	<0.0001
NT-proBNP (pg/mL)	1567.0 (540.5–2599.5)	3624.0 (1888.5–6076.3)	<0.0001
hsCRP (mg/dL)	0.41 (0.21–0.85)	0.81 (0.40–1.81)	<0.0001

hsCRP = high-sensitivity C-reactive protein, MACE = major adverse cardiac event, NT-proBNP = N-terminal pro-B-type natriuretic peptide.

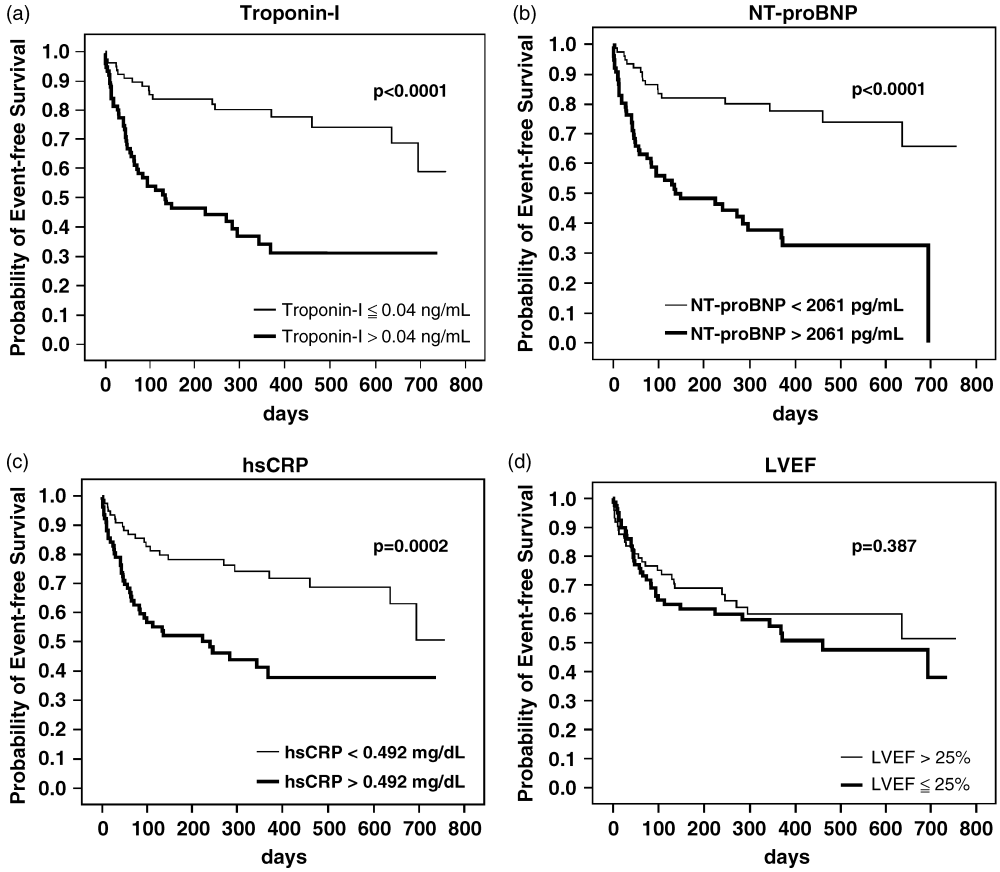

FIG. 1 Kaplan–Meier event probability for study patients stratified into two groups on the basis of median levels of cardiac troponin-I, N-terminal pro-B-type natriuretic peptide (NT-proBNP), high-sensitivity C-reactive protein (hsCRP) and left ventricular ejection fraction (LVEF).

TABLE 3 Multivariate Cox proportional hazard analysis

Variables	Hazard ratios	95% C.I.	p-values
Age > 57 years	1.376	0.713–2.669	0.340
Male gender	0.843	0.407–1.745	0.645
Ischemic heart disease	1.473	0.862–2.517	0.156
Left ventricular ejection fraction ≦ 25%	0.760	0.406–1.423	0.391
Systolic blood pressure < 116 mmHg	2.027	1.063–3.865	0.032
Serum sodium level < 138 mmol/L	2.103	1.116–3.965	0.022
Creatinine clearance < 52 mL/min	1.687	0.823–3.456	0.153
Cardiac troponin I > 0.04 ng/mL	2.283	1.121–4.651	0.023
NT-proBNP > 2061 pg/mL	1.987	1.010–3.796	0.046
hsCRP > 0.492 mg/dL	2.165	1.174–3.993	0.013

hsCRP = high-sensitivity C-reactive protein, NT-proBNP = N-terminal pro-B-type natriuretic peptide.

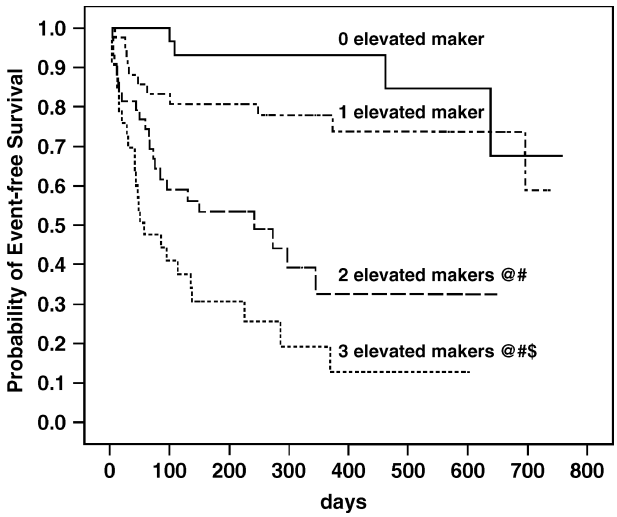

FIG. 2 Kaplan–Meier analyses in study patients stratified by the number of elevated biomarkers. @ $p < 0.0001$, comparisons between patients with two and three elevated biomarkers and those with none; # $p < 0.005$, comparisons between patients with two and three elevated biomarkers and those with one; \$ $p < 0.05$, comparison between patients with two elevated biomarkers and those with three.

death that contribute to the loss of functional myocytes in both ischemic and nonischemic CHF.[1,10–12] Furthermore, BNP and NT-proBNP, part of the neurohormonal axis, are elevated in the setting of left ventricular overload and predict outcome, and are thus major biomarkers of risk.[3,4,10–12] Recent studies also suggest that immune activation and inflammation also play important roles in the pathogenesis and progression of CHF.[5,6] We and others have also reported that an elevated level of hsCRP is an independent predictor of prognosis in CHF.[5,6] To reflect different axes in the pathophysiology of CHF, cTnI, NT-proBNP and hsCRP were chosen as biomarkers in this study.

In the present study, we demonstrated that each biomarker provided independent and incremental prognostic information. Using prospectively defined cut-off points of median levels and categorizing patients by the number of elevated biomarkers, simultaneous assessment of these three pathophysiologically diverse biomarkers enabled powerful prediction of risk of major adverse cardiac events. Even after adjustment for traditional clinical predictors of adverse events, the prognostic value of the multimarker approach remained significant and was more powerful than the single-marker approach. Although this approach lacks quantitative information, and elevations in each biomarker may confer different relative risks for individual components of the composite endpoint, our data shows that this approach is feasible and effective among patients with advanced CHF.

A limitation of the study was the relatively small number of patients included in each group. The low number of patients most likely explains the lack of difference in survival curves when patients were split by median LVEF. However, a statistically significant association was observed between the number of elevated biomarkers and the clinical outcomes in the current study. Furthermore, the study population analyzed here was a high-risk group with a 40% event rate at six months. This group of patients with advanced CHF was not representative of a cross-section of general cardiovascular medicinal practice. The triple marker strategy for risk stratification used in the present study may not be applicable in general clinical practice. Further and larger studies will be required to determine whether this approach is still useful in those patients with much lower risk and earlier stages of CHF. In addition, biomarkers can be influenced by medications.[3,6,11] The ability of treatments to reduce biomarker levels and the prognostic importance thereof require further study. Finally, the biomarkers may interact with each other. As shown in this study, the circulating levels of cTnI and NT-proBNP are correlated with each other. Even though the patients with renal insufficiency may have higher cTnI

and NT-proBNP levels, it is suggested that myocardial loss, secondary to ischemia, may also lead to decrease both in systolic function and in compliance of the left ventricle and to elevation in NT-proBNP. On the other hand, elevation in left ventricular wall stress and NT-proBNP may worsen ischemia and result in myocardial loss.[2–4,7]

Conclusions

Our findings indicate that cTnI, NT-proBNP and hsCRP each provide unique prognostic information in patients with advanced CHF. A simple multimarker strategy that categorizes patients with advanced CHF based on the number of elevated biomarkers is feasible and allows rapid risk stratification in these patients.

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