Mannheim, Germany). The analytical range, intra-assay and interassay coefficients of variation, and normal reference range of the assays were 4.0–4000 pg/ml, 4.94% and 2.22%, and < 18.4 pg/ml, respectively, for BNP; 1.25–250 ng/ml, 5.8% and 1.7%, and < 5.25 ng/ml for H-FABP; and 0.01–25 ng/ml, 1.1% and 1.5%, and < 0.01 ng/ml for cTnT.

Results are presented as mean (SD) for continuous variables. Data were statistically analysed with JMP statistical software (JMP version 5.1, SAS Institute). Differences between groups were estimated by the unpaired t test or Mann–Whitney U test, as appropriate for continuous variables, and by Fisher's exact test or χ^2 test, as appropriate for categorical variables. The risk ratio with the 95% confidence interval for progression to cardiac death, left ventricular assist device or heart transplantation was estimated by univariate and multivariate Cox proportional hazards models. Variables that were significant in univariate analyses were entered into the multivariate analysis. Biochemical values such as BNP and H-FABP were log transformed (ln) to remove skewness of data distribution. Survival curves were constructed by the Kaplan–Meier method and compared by the log rank test. Receiver operating characteristic curves were generated from multiple sensitivity–specificity pairs. A value of $p < 0.05$ was considered significant.

RESULTS

Patient characteristics

During 48 months of follow up, 23 patients had critical cardiac events. Thirteen patients died of left ventricular failure, three patients received a left ventricular assist device and were added to the waiting list for transplantation, and seven patients received a heart transplant. Table 1 compares the characteristics of patients who had critical cardiac events (non-survivors) and the remaining patients (survivors). New York Heart Association functional class, heart rate, BNP and H-FABP concentrations before hospital discharge were significantly higher among non-survivors than among survivors. Left ventricular ejection fraction was significantly lower in non-survivors than in survivors. The two groups did not differ significantly in other variables including drug treatment at discharge.

Table 1 Patients' characteristics

Variable	Non-survivors (n = 23)	Survivors (n = 69)	p Value
Age (years)	50 (13)	49 (11)	0.5519
Men/women	16/7 (70%/30%)	50/19 (72%/28%)	0.7892
NYHA functional class			0.0132
I	1 (4%)	21 (30%)	
II	8 (35%)	26 (38%)	
III	14 (61%)	22 (32%)	
IV	0	0	
Atrial fibrillation	4 (17%)	10 (14%)	0.7375
Duration of CHF (years)	3.6 (2.7)	4.0 (2.4)	0.4979
Body mass index(kg/m^2)	21 (3)	22 (3)	0.2342
Heart rate (beats/min)	81 (13)	73 (12)	0.0176
Mean arterial BP (mm Hg)	81 (10)	82 (11)	0.7447
LVEF (%)	30 (8)	37 (9)	0.0020
LVEDD (mm)	61 (9)	60 (10)	0.4521
QTc (ms)	419 (26)	411 (25)	0.1780
Packed cell volume	0.38 (0.02)	0.38 (0.02)	0.8495
Sodium (mmol/l)	136 (3)	137 (3)	0.5980
Creatinine (μmol/l)	97 (35)	88 (35)	0.8523
Uric acid (μmol/)	488 (184)	428 (143)	0.1816
CK-MB (ng/ml)	5.4 (2.2)	4.7 (2.0)	0.2389
cTnT (ng/ml)	0.02 (0.01)	0.02 (0.01)	0.1155
BNP (pg/ml)	267 (141)	108 (81)	<0.0001
H-FABP (ng/ml)	9.3 (3.5)	5.1 (2.6)	<0.0001
Drugs			
Oral inotropics	3 (13%)	6 (9%)	0.5433
Digitalis	13 (57%)	30 (43%)	0.2776
Nitrates	3 (13%)	12 (17%)	0.6250
Diuretics	22 (96%)	68 (99%)	0.4091
ACE inhibitors	16 (70%)	55 (80%)	0.3154
β blockers	18 (78%)	52 (75%)	0.7778

ACE, angiotensin converting enzyme; BNP, brain natriuretic peptide; BP, blood pressure; CHF, congestive heart failure; CK, creatine kinase; cTnT, cardiac troponin T; H-FABP, heart-type fatty acid binding protein; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

Event analyses

By univariate analyses, functional class ($p = 0.0035$), heart rate ($p = 0.0021$) and left ventricular ejection fraction ($p = 0.0018$) were related to critical cardiac events in DCM. Concentrations of H-FABP(ln) ($p < 0.0001$) and BNP(ln) ($p < 0.0001$) before discharge were also associated with critical cardiac events in DCM. Among five significant variables in univariate analysis, H-FABP(ln) and BNP(ln) concentrations were the sole independent predictors of critical cardiac events in patients with DCM (table 2). Repeating the analysis with these two independent variables showed that H-FABP(ln) ($p = 0.0032$ and BNP(ln) ($p = 0.0001$) had significant effects on critical cardiac events. Risk ratios of H-FABP(ln) and BNP(ln) were 7.450 and 10.87, respectively, in this reanalysis. Thus, patients had a 10.9 times higher risk of events with each increase of BNP(ln) by one unit. Likewise, patients had a 7.5 times higher risk of events with each increase of H-FABP(ln) by one unit. [1]

Figure 1 shows Kaplan–Meier event curves according to the median concentrations of cTnT (0.02 ng/ml), BNP (138 pg/ml) and H-FABP (5.4 ng/ml). Patients with a concentration of cTnT ≥ 0.02 ng/ml had a similar survival rate to those with cTnT < 0.02 ng/ml (log rank test, $p = 0.1585$). Patients with BNP ≥ 138 pg/ml had a significantly lower survival rate than those with BNP < 138 pg/ml (log rank test, $p = 0.0008$). Patients with H-FABP ≥ 5.4 ng/ml had a significantly lower survival rate than those with H-FABP < 5.4 ng/ml (log rank test, $p < 0.0001$). The area under the receiver operating characteristic curve for critical cardiac events was similar between H-FABP and BNP (0.853 *v* 0.848, $p = 0.9322$). Thus, the prognostic value of the H-FABP concentration was comparable to that of the BNP concentration. When the H-FABP and BNP concentrations were combined to produce four segments (H-FABP ≥ 5.4 ng/ml and BNP < 138 pg/ml; H-FABP ≥ 5.4 ng/ml and BNP ≥ 138 pg/ml; H-FABP < 5.4 ng/ml and BNP < 138 pg/ml; H-FABP < 5.4 ng/ml and BNP ≥ 138 pg/ml) in the study population, patients with H-FABP ≥ 5.4 ng/ml and BNP ≥ 138 pg/ml had a lower survival rate (log rank test, $p = 0.0002$) (fig 1D).

DISCUSSION

In the present study, we showed that a serum concentration of H-FABP before discharge independently predicted the long-term risk of critical cardiac events in non-ischaemic

Table 2 Multivariate proportional hazards analysis

Variable	RR	95% CI	p Value
NYHA class II v I	1.971	0.421 to 5.825	0.3190
NYHA class III v I	3.051	0.636 to 9.736	0.1344
Heart rate	1.025	0.978 to 1.076	0.3022
LVEF	0.957	0.898 to 1.017	0.1601
BNP(ln)	10.87	3.527 to 35.32	<0.0001
H-FABP(ln)	7.450	1.722 to 36.12	0.0068

BNP, brain natriuretic peptide; CI, confidence interval; H-FABP, heart-type fatty acid binding protein; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; RR, risk ratio.

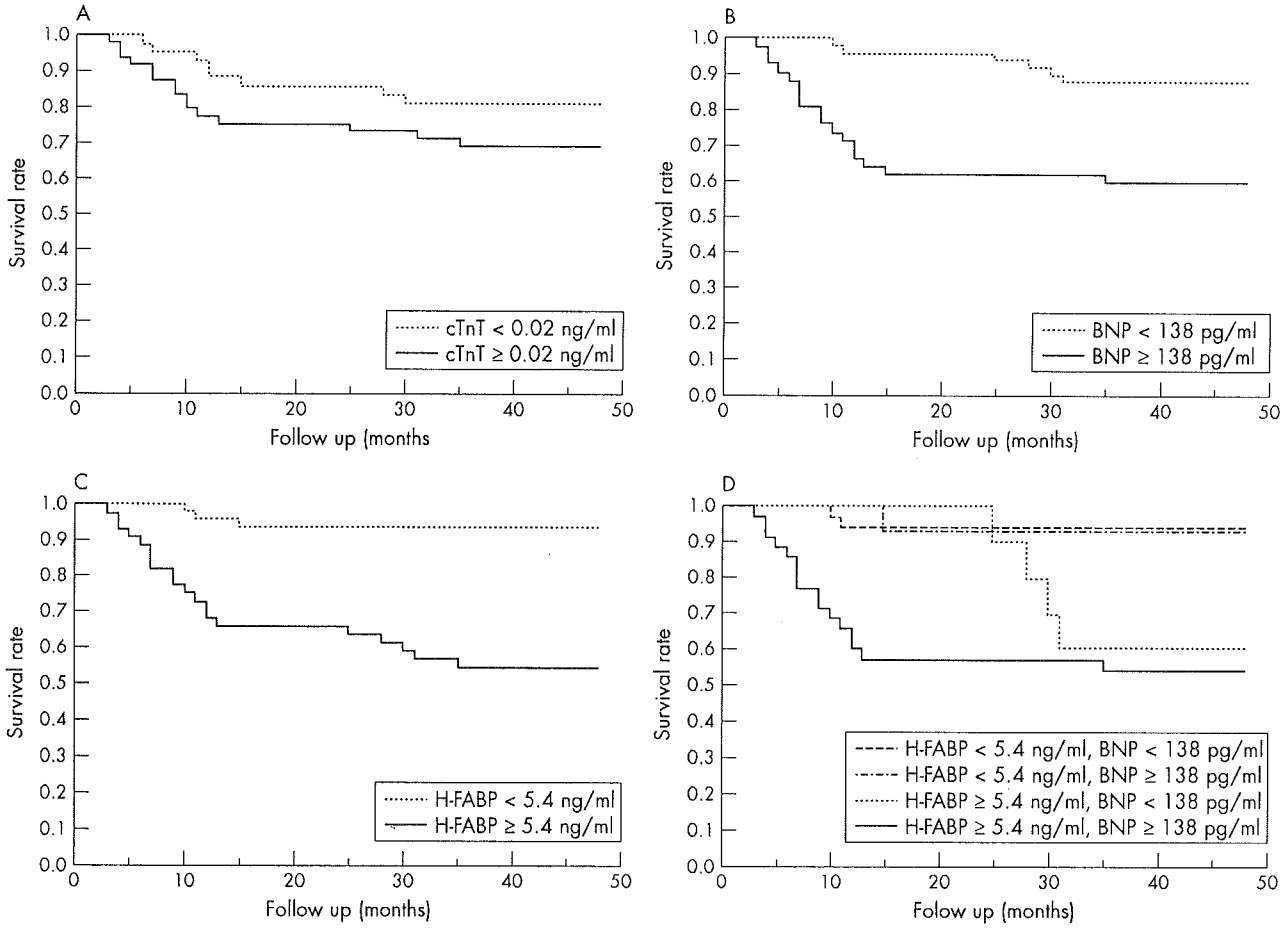

Figure 1 Kaplan–Meier event curves according to the median concentrations of (A) cardiac troponin T (cTnT), (B) brain natriuretic peptide (BNP), (C) heart-type fatty acid binding protein (H-FABP) and (D) H-FABP and BNP combined.

DCM. The predictive power of H-FABP was comparable to that of BNP. Furthermore, a combination of high-concentration BNP and high-concentration H-FABP yielded a worse prognosis.

cTnT concentrations were reported to rise in DCM[15] as well as in acute myocardial infarction.[16] The cut off value of 0.02 ng/ml in the present study was the same as that in a previous report on DCM.[15] cTnT is located in myofilaments, and its molecular weight (37.0 kDa) is greater than that of H-FABP (14.9 kDa), found in cytosol, which makes cTnT harder to detect than H-FABP. In fact, cTnT was detected in 36–46% of patients with acute myocardial infarction,[16 17] whereas H-FABP was detected in 93%.[10] In the present study, the concentrations of cTnT were similar between survivors and non-survivors. Two Kaplan–Meier event curves for patients over and under the cut off did not differ significantly. A sustained rise of cTnT for 16 months significantly and independently predicted adverse outcomes in DCM.[15] We assume that a point-of-care measurement of cTnT at a single time point may not closely reflect the severity of non-ischaemic DCM. A previous report on the predictability of cTnT for cardiac events in heart failure may be attributable to the ischaemic aetiology of heart failure.[18]

Ongoing myocardial damage in DCM may be one of the plausible mechanisms for the release of H-FABP.[19] The correlation between H-FABP concentration and heart failure severity, and the correlation between H-FABP concentration and BNP concentration were reported in a previous study.[19] Although that previous study[19] suggested that the prognostic power of H-FABP for cardiac events in DCM is comparable to that of BNP, we confirmed the role of H-FABP as a predictor in our four-year follow up. In the present study, an endomyocardial biopsy did not provide evidence of overt active myocarditis in all of the patients. Our method did not thoroughly exclude the possibility of inactive and chronic inflammatory or viral cardiomyopathy causing non-ischaemic cardiomyopathy.[20] For any reason, a transient loss of cell membrane integrity may cause cytoplasmic molecules to leak into the bloodstream. These events may yield detectable biomarkers even in the absence of myocyte death. Although the present study did not identify known possible causes of non-ischaemic heart failure, such as chronic myocardial inflammation or chronic viral infection,[1 21] increased serum concentrations of H-FABP were shown to predict the long-term risk of critical cardiac events with a predictive power comparable to that of BNP, independently of the underlying causes. In this view, H-FABP may provide additional information for risk stratification and management of these patients with DCM. Whereas raised H-FABP concentrations reflect myocardial membrane damage, raised BNP concentrations reflect increased ventricular filling pressure. The combination of these two provides an index for a worse prognosis. Thus, H-FABP concentration may provide a novel estimate of the clinical outcome in DCM. Caution is needed in interpreting the present small study, which may have confounding associations of other variables. Thus, larger clinical trials would help to clarify the potential role of H-FABP in determining the prognosis of patients with DCM.

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REFERENCES

1 **Dec GW**, Fuster V. Idiopathic dilated cardiomyopathy. *N Engl J Med* 1994;**331**:1564–75.
2 **Remme WJ**, Swedberg K. Task Force for the Diagnosis and Treatment of Chronic Heart Failure, European Society of Cardiology. Guidelines for the diagnosis and treatment of chronic heart failure. *Eur Heart J* 2001;**22**:1527–60.
3 **Troughton RW**, Frampton CM, Yandle TG, *et al.* Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. *Lancet* 2000;**355**:1126–30.
4 **Nakagawa O**, Ogawa Y, Itoh H, *et al.* Rapid transcriptional activation and early mRNA turnover of brain natriuretic peptide in cardiocyte hypertrophy: evidence for brain natriuretic peptide as an "emergency" cardiac hormone against ventricular overload. *J Clin Invest* 1995;**96**:1280–7.
5 **Schirmer H**, Omland T. Circulating N-terminal pro-atrial natriuretic peptide is an independent predictor of left ventricular hypertrophy in the general population. The Tromso study. *Eur Heart J* 1999;**20**:755–63.
6 **Buckley MG**, Sethi D, Markandu ND, *et al.* Plasma concentrations and comparisons of brain natriuretic peptide and atrial natriuretic peptide in normal subjects, cardiac transplant recipients and patients with dialysis-independent or dialysis-dependent chronic renal failure. *Clin Sci (Lond)* 1992;**83**:437–44.
7 **Sabatine MS**, Morrow DA, de Lemos JA, *et al.* Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. *Circulation* 2002;**105**:1760–3.
8 **Ohta Y**, Watanabe K, Nakazawa M, *et al.* Carvedilol enhances atrial and brain natriuretic peptide mRNA expression and release in rat heart. *J Cardiovasc Pharmacol* 2000;**36**:S19–23.
9 **Schaap FG**, van der Vusse GJ, Glatz JF. Fatty acid-binding proteins in the heart. *Mol Cell Biochem* 1998;**180**:43–51.
10 **Okamoto F**, Sohmiya K, Ohkaru Y, *et al.* Human heart-type cytoplasmic fatty acid-binding protein (H-FABP) for the diagnosis of acute myocardial infarction: clinical evaluation of H-FABP in comparison with myoglobin and creatine kinase isoenzyme MB. *Clin Chem Lab Med* 2000;**38**:231–8.
11 **Richardson P**, McKenna W, Bristow M, *et al.* Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. *Circulation* 1996;**93**:841–2.
12 **Aretz HT**, Billingham ME, Edwards WD, *et al.* Myocarditis: a histopathologic definition and classification. *Am J Cardiovasc Pathol* 1987;**1**:3–14.
13 **Edwards WD**, Holmes DR Jr, Reeder GS. Diagnosis of active lymphocytic myocarditis by endomyocardial biopsy: quantitative criteria for light microscopy. *Mayo Clin Proc* 1982;**57**:419–25.
14 **Katsuragi M**, Yutani C, Imakita M, *et al.* Cell infiltration caused deterioration in the prognosis of patients with clinical diagnosis of dilated cardiomyopathy (DCM): application of biopsy criteria of myocarditis to 42 autopsy cases. *Heart Vessels* 1993;**8**:42–7.
15 **Sato Y**, Yamada T, Taniguchi R, *et al.* Persistently increased serum concentrations of cardiac troponin t in patients with idiopathic dilated cardiomyopathy are predictive of adverse outcomes. *Circulation* 2001;**103**:369–74.
16 **Giannitsis E**, Muller-Bardorff M, Lehrke S, *et al.* Admission troponin T level predicts clinical outcomes, TIMI flow, and myocardial tissue perfusion after primary percutaneous intervention for acute ST-segment elevation myocardial infarction. *Circulation* 2001;**104**:630–5.
17 **Ohman EM**, Armstrong PW, Christenson RH, *et al.* Cardiac troponin T levels for risk stratification in acute myocardial ischemia. GUSTO IIA Investigators. *N Engl J Med* 1996;**335**:1333–41.
18 **Ishii J**, Nomura M, Nakamura Y, *et al.* Risk stratification using a combination of cardiac troponin T and brain natriuretic peptide in patients hospitalized for worsening chronic heart failure. *Am J Cardiol* 2002;**89**:691–5.
19 **Goto T**, Takase H, Toriyama T, *et al.* Circulating concentrations of cardiac proteins indicate the severity of congestive heart failure. *Heart* 2003;**89**:1303–7.
20 **Maisch B**, Ristic AD, Hufnagel G, *et al.* Dilated cardiomyopathies as a cause of congestive heart failure. *Herz* 2002;**27**:113–34.
21 **Frustaci A**, Chimenti C, Calabrese F, *et al.* Immunosuppressive therapy for active lymphocytic myocarditis: virological and immunologic profile of responders versus nonresponders. *Circulation* 2003;**107**:857–63.

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