sets of primers were designed for each gene. The primer set with most efficient amplification of a target cDNA by PCR was selected for final use, which was estimated by conventional electrophoresis and ethidium-bromide staining. Subsequently, the TaqMan probe inherent to each primer set was prepared, which is an oligonucleotide labelled with a reporter dye (FAM) at the 5′-end and a quencher dye (TAMRA) at the 3′-end. The nucleotide sequences of the primers and corresponding TaqMan probes used in this study are shown in *Table 2*.

Real-time RT–PCR

The amount of cDNA of interest was analysed using ABI 7700 Prism Sequence detection system (Applied Biosystems), which was based on RT-PCR. The reaction solution was assembled in a volume of 25 μL, which comprised Platinum Quantitative PCR Super Mix-UDG (Invitrogen), forward and reverse primers (final concentration 300 nM each), TaqMan probe (final concentration 200 nM), and cDNA mixture (about 10 ng). The conditions for RT–PCR were preheating at 50°C for 2 min and at 95°C for 10 min, followed by 40 cycles of shuttle heating at 95°C for 15 s and at 60°C for 1 min. We used normal heart samples commercially available as normal controls. From human heart total RNA (Invitrogen), cDNA mixture was synthesized and used as a normal standard (normal-1) to generate the working standard for quantification of the cDNA of interest, which plots the relationship between the dilution of the standard cDNA mixture and the corresponding Ct value (the number of cycles necessary to obtain a threshold fluorescent signal). The initial quantity of the cDNA of interest, in a certain cDNA mixture, was calculated from the working standard and then normalized to that of GAPDH determined with Pre-developed TaqMan Assay Reagent Endogenous Control™ (Applied Biosystems). The normalized value for each target cDNA is considered to reflect the mRNA expression levels of the corresponding gene in a test sample relative to normal-1. The quantitative assay for each cDNA was performed at least twice in triplicate and statistically analysed. In order to test the validity of normal-1 cDNA as normal control, cDNA mixtures were also synthesized from the other commercially available total RNAs from normal hearts (Clontech and Origen technologies). Changes in the mRNA expression levels by β-blocker treatment were expressed by the ratio of post- to pre-values.

Table 2 Primers and probes

ACE	
Sense	5′-GTGGAGGAATATGACCGGACAT-3′
Antisense	5′-TGTTGGTGTTGTAGTTCCAGTTGG-3′
Probe	5′-AGGTGGTGTGGAACGAGTATGCCGAG-3′
ATNG	
Sense	5′-CCAGGACAACTTCTCGGTGACT-3′
Antisense	5′-CATAGTGAGGCTGGATCAGCAG-3′
Probe	5′-AAGTGCCCTTCACTGAGAGCGCCTG-3′
AT_1	
Sense	5′-GATACCTGGCTATTGTTCACCCA-3′
Antisense	5′-GCAGGTGACTTTGGCTACAAGC-3′
Probe	5′-AAGTCCCGCCTTCGACGCACAA-3′
MR_1	
Sense	5′-ACAGCACTGGTTCCTCAGCTC-3′
Antisense	5′-GAGCTGTCATAGCCTGCATATACAA-3′
Probe	5′-ACCTTCCCCCGTTATGGTCCTTGAAAAC-3′
TGF-β_1	
Sense	5′-CCAGCATCTGCAAAGCTCC-3′
Antisense	5′-GGTCCTTGCGGAAGTCAATGT-3′
Probe	5′-CACCAACTATTGCTTCAGCTCCACGGA-3′
HGF	
Sense	5′-CAAATGTCAGCCCTGGAGTTC-3′
Antisense	5′-GGTCTTTACCCCGATAGCTCG-3′
Probe	5′-TGATACCACACGAACACAGCTTTTTGCC-3′
Col I	
Sense	5′-GCTACCCAACTTGCCTTCATG-3′
Antisense	5′-GCTGTTCTTGCAGTGGTAGGTG-3′
Probe	5′-TGCTGGCCAACTATGCCTCTCAGAACA-3′
Col III	
Sense	5′-CCCACTATTATTTTGGCACAACAG-3′
Antisense	5′-GCATGGTTCTGGCTTCCAGA-3′
Probe	5′-TCCCATCTTGGTCAGTCCTATGCGGA-3′

ATNG, angiotensinogen; MR_1, mineralcorticoid Type I receptor.

Immunoblotting

From the right ventricular muscle biopsied in six patients, whole homogenate protein as well as total RNA were extracted. The myocardial samples were homogenized in 1.0 mL ISOGEN™ reagent (Nippon Gene), thoroughly mixed with 0.2 mL chloroform, and centrifuged at 15 000 *g* for 15 min at 4°C. The interphase and organic phase were mixed with 0.3 mL ethanol and centrifuged at 2000 *g* for 5 min at 4°C. The supernatant was transferred into a micro test tube, mixed with 1.5 mL isopropanol, and centrifuged at 12 000 *g* for 10 min at 4°C. The precipitate was mixed with 2 mL 0.3 M guanidine hydrochloride in 95% ethanol, stored for 20 min at room temperature, and centrifuged at 7500 *g* for 5 min at 4°C. After the process was repeated three times, the precipitate with 2 mL ethanol was centrifuged at 7500 *g* for 5 min at 4°C. The precipitated total protein was air-dried and dissolved in sodium dodecyl sulfate (SDS) solution. The integrities of extracted proteins were examined by SDS-polyacrylamide gel electrophoresis (PAGE) with silver staining. Extracted proteins were loaded onto 15% SDS-polyacrylamide gel and separated. Proteins were electrotransferred onto nitrocellulose membrane (Invitrogen) and blocked with 1% Block Ace (Serotec, Oxford, UK) in phosphate-buffered saline Tween-20 (PBS-T) at 4°C. After washed with PBS-T, the membrane was exposed for 1 h at room temperature to monoclonal antibody against TGF-β_1 (MAB1032, CHEMICON, Temecula, CA, USA) in PBS-T at a dilution of 1:150. The membrane was washed with PBS-T and incubated along with a horseradish peroxidase (HRP)-conjugated donkey anti-mouse secondary antibody (715-035-151, Jackson Immuno Research, Baltimore, PA, USA) for 1 h in PBS-T at a dilution of 1:50 000. After the membrane was washed with PBS-T, the immunological detection of TGF-β_1 protein was performed with ECL Plus (Amersham, Chicago, IL, USA) and the band density was captured and degitalized by EDAS290 (Eastman Kodak, Rochester, NY, USA). The expression levels of TGF-β_1 protein were normalized to that of actin. The amount of TGF-β_1 protein was determined relative to the mean amount of the samples.

Statistical analysis

The sample size was obtained as follows: the primary variable was the mRNA level of Col I or III and the sample size was based on a two-sided paired *t*-test with a significance level of 0.05, a power level of 0.80, and with an anticipated effect size d = difference of means/standard deviation = 0.75. The required sample size was 16. We decided 17 as sample size for avoiding the lack of required sample size during the follow-up.

Changes in gene expressions by the β-blockers were compared by the two-sided paired *t*-test. The relationship between gene expression and clinical indexes was determined by linear regression analysis. A value of $P < 0.05$ was considered significant. Data are expressed as mean ± SD.

Table 3 Changes in the expression of factors involved in renin-angiotensin system

	ACE	AGTN	AT_1	MR_1	TGF-β_1	HGF	Col I	Col III
DCM								
Before	1.02 ± 0.53	0.83 ± 0.46	0.30 ± 0.17	0.83 ± 0.30	0.64 ± 0.33	0.76 ± 0.31	1.08 ± 0.72	2.06 ± 1.81
After	1.12 ± 0.44	0.84 ± 0.48	0.36 ± 0.24	0.90 ± 0.36	0.55 ± 0.22	0.87 ± 0.64	0.65 ± 0.26	1.05 ± 0.74

Expression levels of each gene are expressed as the relative value to those in the normal control. AGTN, angiotensinogen; MR_1, mineralcorticoid Type I receptor; DCM, dilated cardiomyopathy; Before, before 4 months treatment with β-blocker; After, after 4 months treatment with β-blocker.

Results

Clinical indices of chronic heart failure

End-diastolic volume index (EDVI) (153 ± 32 vs. 113 ± 23 mL/m^2, $P = 0.0007$), left ventricular ejection fraction (LVEF) (21 ± 7 vs. 35 ± 9%, $P < 0.0001$), plasma BNP concentration (165 ± 146 vs. 45 ± 79 pg/mL, $P = 0.0009$), and WR (53 ± 14 vs. 42 ± 13%, $P = 0.002$) significantly improved 4 months after the administration of β-blockers.

Myocardial gene expression

The expressions of genes that belong to renin-angiotensin system before the β-blocker treatment in DCM patients were close to that in normal controls except for angiotensin II Type 1 receptor (*Table 3*). The expression level of angiotensin II Type 1 receptor was down-regulated (0.30 ± 0.17) compared with that of normal control.

The mRNA expression levels of both Col I (1.08 ± 0.72 vs. 0.65 ± 0.26, $P = 0.024$) and Col III (2.06 ± 1.81 vs. 1.05 ± 0.74, $P = 0.018$) were significantly reduced by the β-blocker (*Figure 1*). The expression levels of either Col I or Col III did not correlate with EDVI, LVEF, or plasma BNP concentrations. Nevertheless, both Col I ($r = 0.560$, $P = 0.041$) and Col III ($r = 0.630$, $P = 0.008$) expressions correlated with WR before the β-blocker treatment, (*Figure 2A* and *B*). The expression levels of Col I ($r = 0.662$, $P = 0.032$) correlated with that of TGF-β_1 before the β-blocker treatment. After the β-blocker treatment, either Col I or Col III expressions did not correlate with WR. However, the extent of changes in Col I ($r = 0.542$, $P = 0.036$) mRNA expression was positively correlated with that in WR. The extent of changes in Col III ($r = 0.455$, $P = 0.089$) mRNA expression has tendency of correlation with that in WR.

The extent of changes in both Col I ($r = 0.813$, $P < 0.0001$) and Col III ($r = 0.619$, $P < 0.0001$) mRNA expressions were positively correlated with that in TGF-β_1 (*Figure 3A* and *B*). Moreover, the extent of changes in TGF-β_1 ($r = 0.606$, $P = 0.002$) mRNA expression correlated with that in WR (*Figure 4*), although it did not correlate with that of the other components of renin-angiotensin system.

The expression levels of HGF correlated with that of mineral corticoid receptor 1 both before and after 4 months treatment with the β-blocker (*Figure 5A* and *B*). The extent of changes in HGF mRNA expression was also correlated with that in mineralcorticoid Type 1 receptor mRNA expression (*Figure 5C*).

Protein expression

The integrities of extracted proteins were confirmed by SDS-PAGE with silver staining (*Figure 6A*). The expression

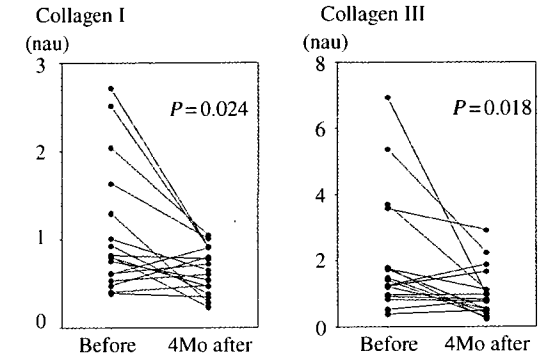

Figure 1 Changes in collagen mRNA expression levels 4 months after the treatment with β-blockers. The abundance of each mRNA expression in DCM patients is expressed by the relative value to that in the normal control. Before, before the β-blocker therapy, 4Mo after, 4 months after the treatment with β-blocker, nau, normalized arbitrary unit.

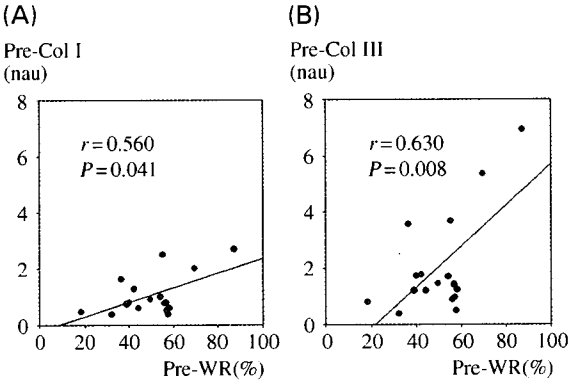

Figure 2 Relation between collagen mRNA expression and WR before the β-blocker therapy. The expression level of collagen in DCM patients is expressed by the relative value to that in the normal control. Pre, before the β-blocker therapy, nau, normalized arbitrary unit.

level of TGF-β_1 protein was analysed by immunoblotting. We observed bands corresponding to TGF-β_1 protein at 12.5 kDa in each lane (*Figure 6B*). The β-blocker reduced the protein expression levels of TGF-β_1 (1.15 ± 0.64 vs. 0.85 ± 0.62, $P = 0.017$) as well as the mRNA expression levels (*Figure 6C*).

Discussion

We found that, in the failing hearts of mild to moderate degrees, the gene expression level of collagen correlated not only with that of TGF-β_1, but also with the sympathetic nerve activity. The collagen mRNA expression was decreased 4 months after the onset of the treatment with the

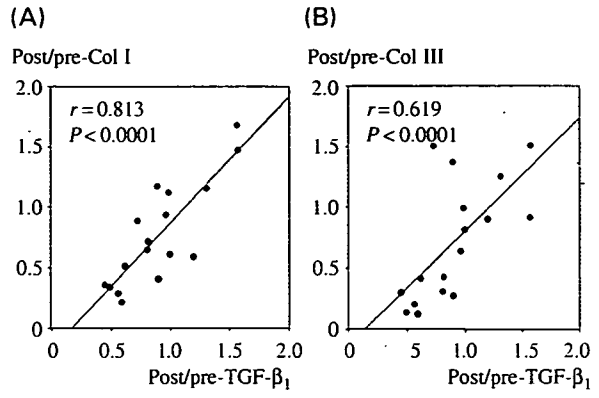

Figure 3 Relation between changes in collagen mRNA expression and TGF-β_1 mRNA expression by β-blocker therapy. Post/pre, the ratio of the value after to before the treatment with β-blocker.

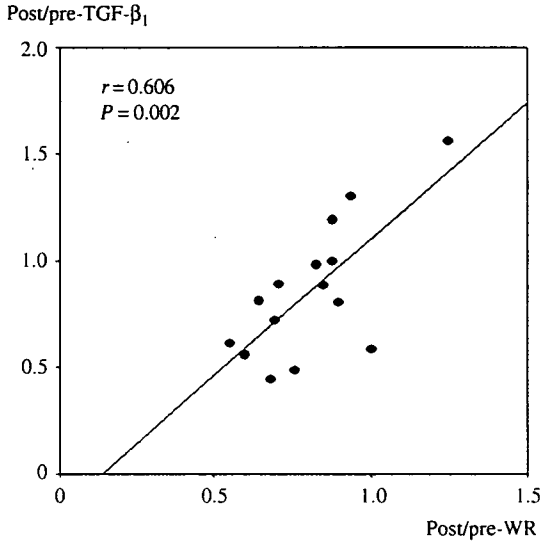

Figure 4 Relation between changes in the expression level of TGF-β_1 and the value of WR. Post/pre, the ratio of the value after to before the treatment with β-blocker.

β-blocker. Changes in collagen mRNA expression were positively correlated with those in TGF-β_1, closely related to changes in cardiac sympathetic nerve activity by the β-blocker treatment.

Expression of the genes related to renin–angiotensin system in DCM patients

Although ACE mRNA in this study did not show significant increase, ACE mRNA is reported to increase in patients with aortic stenosis and aortic regurgitation using right ventricular biopsy samples,[19] or in patients with advanced heart failure using excised heart.[20] This difference may be attributed to the difference of the severity of heart failure, because New York Heart Association (NYHA) functional classes in this study were mild to moderate. The down-regulation of cardiac angiotensin II Type 1 receptor mRNA was observed in this study, consistently with the previous report,[21] which may reflect an increase in tissue angiotensin II content. As ACE and angiotensinogen mRNA expression did not increase significantly, the down-regulation of cardiac angiotensin II Type 1 receptor mRNA may suggest that ACE activity is enhanced even in patients with mild to moderate severity of heart failure.

Regulation of collagen synthesis

Several studies targeted at DCM have demonstrated changes in the collagen content both at the protein[7,22-24] or mRNA level,[7] characterized primarily by an accumulation of Col I. Indeed, the mRNA levels of both Col I and Col III mRNAs increased in some patients in this study. Different extent of increases in Col I and Col III contributes an increased Col I/Col III ratio in the myocardium of DCM patients.[7] However, the relative increase in the mRNA level of Col III to I in this study seems to be inconsistent with these previous findings. We did not clarify this disparity. One explanation is that the ratio may be different between the stages or extent of progression of pathophysiological states of DCM.

Norepinephrine induces the accumulation of collagen in the myocardium independent of haemodynamic changes

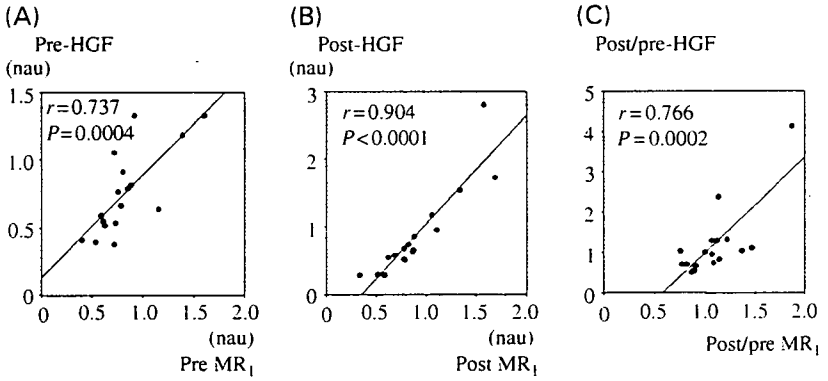

Figure 5 Relation between the expression levels of HGF and MR_1. The abundance of each mRNA expression in DCM patients is expressed by the relative value to that in the normal control. Post/pre, the ratio of the value after to before the treatment with β-blocker. MR_1, mineralcorticoid Type 1 receptor, nau, normalized arbitrary unit.

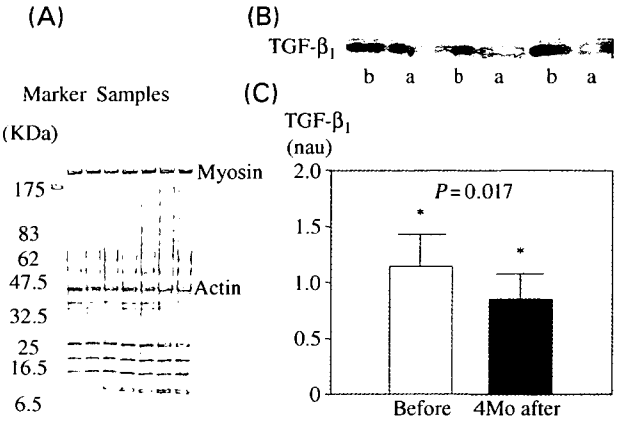

Figure 6 (*A*) Representative SDS-PAGE with silver staining in extracted whole homogenate protein. (*B*) Representative immunoblot for TGF-β_1 in failing hearts before (b) and after (a) the β-blocker therapy. (*C*) Summarized data from immunoblot analysis of TGF-β_1. Before, before the β-blocker therapy, 4Mo after, 4 months after the treatment with β-blocker, nau, normalized arbitrary unit.

produced by norepinephrine,[25] and was attributed to a direct effect of norepinephrine on cardiac adrenoreceptor.[12] We could show that cardiac sympathetic nerve activity represented by WR of MIBG correlated with the Col I and Col III mRNA expression. This result, in combination with the results of the previous studies, provides one aspect of the theoretical background of the β-blocker treatment for chronic heart failure.

The increase in collagen synthesis by norepinephrine is reported to be accompanied by the increase in TGF-β_1 mRNA abundance in rats heart.[13] In addition to catecholamines, activation of renin-angiotensin system is also believed to play an important role in the collagen metabolism. A primary mediator of angiotensin II effects on collagen metabolism is also thought to be TGF-β_1, because it has been shown to stimulate collagen synthesis *in vitro*,[26] and activates a wide array of processes that collectively increase extracellular matrix production.[27] Proteins and mRNA of TGF-β_1 are increased in patients with aortic stenosis and regurgitation.[9] In DCM patients, TGF-β_1 mRNA expression was not increased significantly.[7] TGF-β_1 mRNA in this study was decreased compared with normal control. When myocardial fibrosis progresses in the early stage of DCM, the expression of TGF-β_1 is up-regulated. However, at the end-stage of DCM, the expression of TGF-β_1 may be down-regulated even when the TGF-β_1 protein is accumulated in the myocardium as was observed in the present study. Functional classes of patients were stabilized mild to moderate by conventional therapy, but most of the patients had relative severe DCM and long clinical course before the catheterization. Therefore, basal levels of the expression of TGF-β_1 may be already down-regulated. Importantly, the treatment with the β-blocker further reduced the expression level of TGF-β_1 in the present study, suggesting that the small amount of progression of fibrosis may contribute to the progression of disease states.

The effects of β-blockers on the expression of collagen mRNA expression

The β-blockers are reported to reduce collagen production in rat heart with left ventricular dysfunction.[14,15] This study, to our best of our knowledge, is the first to have investigated the effect of β-blockers on the collagen mRNA expression in DCM patients. This effect may contribute to the effect of β-blockers of left ventricular reverse remodelling,[28] in which the function of cardiac fibroblast exerts an important role.[6]

Results of this study may provide some useful information to elucidate the mechanism of the decreases in collagen mRNA abundance by the β-blocker treatment. The extent of changes in Col I mRNA abundance, tended to correlate with the changes in WR and correlated with TGF-β_1 mRNA abundance by the β-blocker treatment, which again implies that both cardiac sympathetic nerve activity and TGF-β_1 was largely related to the collagen synthesis. Because the changes in TGF-β_1 was associated with that in WR, the decrease in collagen synthesis by β-blocker may be mediated by TGF-β_1, which is consistent with previous report.[13]

HGF is reported to attenuate collagen synthesis or promote collagen degradation.[10,29] However, HGF did not seem to play the major role for the reduction of collagen by the β-blocker treatment, because HGF mRNA expression was irrelevant to collagen mRNA expression. HGF mRNA expression, in contrast, closely correlated with mineralcorticoid receptor Type 1 mRNA expression, which suggests a close relationship between tissue aldosterone concentration and HGF production. The combination treatment with β-blocker and spironolactone is expected to be more effective than the monotherapy in respect of the inhibition of collagen synthesis.

The dose of β-blocker and clinical improvement

We observed the marked improvements of cardiac function despite a low dose of β-blocker. Indeed, the target dose of β-blocker in the present study was less than that used in other clinical trials.[30-33] However, a low dose of β-blocker is also reported to improve left ventricular function and increase survival rates in patients with congestive heart failure.[34,35] On the other hand, we cannot deny the possibility that some patients could improve cardiac function spontaneously or uncommonly during the study period. Another possibility for the effectiveness of a low-dose β-blocker is that all patients in this study were DCMs without coronary artery disease, and there is a tendency for more improvement in left ventricular function in the non-ischaemic patient than in the ischaemic patient by carvedilol.[34] Since we did not set the control group for ethical reasons, we cannot perform the quantitative analysis of the improvements of cardiac function, although the cardiac improvements due to a β-blocker in the present study seem to be remarkable compared with the control data that have been already published.[30-35]

Study limitations

Out of 34 referred patients, 17 were not included for various reasons. The exclusion of patients based on our criteria, after the start of the study, could introduce selection bias into the results.

Because of the lack of control groups without the use of β-blockers, changes in mRNA expression cannot be solely attributable to the effects of β-blockers. Moreover, because of the small sample size, the results of this study

must be interpreted with caution. However, the decrease in collagen mRNA expression after β-blocker treatment in this study is consistent with the results examined in rat hearts with chronic pressure overload.[15] By extrapolating from these results, we may attribute the changes in other gene expressions in this study to the effects of β-blockers.

GAPDH is stably expressed in human myocardium from patients with various cardiac conditions, thereby validating their use as a reference gene for internal normalization in the study using small biopsy samples. Although previous reports have documented variations in GAPDH gene expression in circumstances such as hypoxia,[36] other studies state that GAPDH is unaltered in heart failure and GAPDH is usually used as reference gene in real-time RT-PCR for not only non-failing heart but also failing heart.[37,38] Further study is required to validate using GAPDH as a reference gene because it is not determined definitely whether or not there is a change in the absolute abundance of GAPDH after β-blocker treatment.

We added β-blockers on conventional therapy including ACE-inhibitors, which had been administered at least 1 month before the beginning of β-blocker treatment. ACE-inhibitors or the combination of β-blockers with ACE-inhibitors might be partially responsible for some of these changes in gene expression, because the observation time in the present study may not be enough to confirm that the remodelling effect is only due to the β-blocker and not to the ACE-inhibitors.

Conclusions

The collagen mRNA expression, which is related to the cardiac sympathetic nerve activity, is inhibited by the treatment with β-blockers. This inhibition seems to be mediated by cardiac TGF-β_1. The clinical benefit of β-blockers can be partly explained by the effect of β-blockers on this collagen metabolism.

Acknowledgements

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Conflict of interest: none declared.

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CARDIOVASCULAR MEDICINE

Heart-type fatty acid binding protein is a novel prognostic marker in patients with non-ischaemic dilated cardiomyopathy

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Objective: To determine whether concentrations of heart-type fatty acid binding protein (H-FABP) measured before hospital discharge predict critical cardiac events in patients with idiopathic dilated cardiomyopathy (DCM).
Patients: 92 consecutive patients with DCM were enrolled and followed up for four years.
Main outcome measures: Serum concentrations of H-FABP, brain natriuretic peptide (BNP), cardiac troponin T before hospital discharge and survival rate.
Results: 23 patients died of cardiac causes, received a left ventricular assist device or underwent heart transplantation during the four-year follow up. Univariate analyses showed that New York Heart Association functional class, heart rate, ejection fraction, serum H-FABP and plasma BNP were significant variables. According to multivariate analysis, serum H-FABP and plasma BNP concentrations were independent predictors of critical cardiac events. Cardiac troponin T before hospital discharge was not a predictor. The area under the receiver operating characteristic curve for death from critical cardiac events was similar between H-FABP and BNP. Patients with an H-FABP concentration at or above the median (≥ 5.4 ng/ml) had a significantly lower survival rate than those below the median, according to analysis by log rank test ($p < 0.0001$). When combined with BNP concentration at or above the median (≥ 138 pg/ml), H-FABP below the median predicted the worst prognosis among the combinations.
Conclusions: The concentration of serum H-FABP before discharge from hospital may be an independent predictor for critical cardiac events in DCM.

Idiopathic non-ischaemic dilated cardiomyopathy (DCM) has wide range of phenotypes and variable clinical outcomes.[1] Identification of patients with DCM at higher risk for adverse outcomes in its earlier stage may optimise the use of limited health care resources. Brain natriuretic peptide (BNP) is now widely recognised as the most powerful prognostic marker for heart failure,[2] and BNP-guided tailored treatment[3] is advocated. Raised BNP in heart failure is mainly attributable to its gene expression in stretched cardiomyocytes undergoing raised ventricular pressure.[4] The regulation of BNP secretion, however, is complex. BNP is also raised in patients with cardiac hypertrophy,[5] renal failure[6] or acute coronary syndrome.[7] β blocker directly enhances expression and release of BNP from cardiomyocytes.[8]

Fatty acid binding protein is extremely abundant in cytoplasm, has low molecular weight and is considered to be one of the key fatty acid carrier proteins.[9] Thus, fatty acid binding proteins are rapidly released into the circulation shortly after cell damage. Heart-type fatty acid binding protein (H-FABP) is immunologically specific to cardiomyocytes and is used as an early diagnostic marker for acute myocardial infarction.[10] In the present study, we tested whether the concentrations of H-FABP measured before discharge predict critical cardiac events for patients with DCM.

METHODS

Of 97 consecutive patients with DCM admitted to our institute for diagnosis or treatment between January 1997 and December 2000, five patients were excluded from this study because of renal dysfunction (serum creatinine concentration ≥ 177 μmol/l). The remaining 92 patients (66 men and 26 women; mean age 49 years, range 16–76 years) were enrolled in the study. The study procedures were in accordance with the guidelines of our institute, and informed consent was obtained from each patient. The diagnosis of DCM was based on the definition of the World Health Organization/International Society and Federation of Cardiology Task Force.[11] No patient had a history of myocardial infarction, infective myocarditis, metabolic disease or systemic illness. All patients underwent coronary angiography and endomyocardial biopsy for differential diagnosis of DCM. No significant coronary stenosis was found in any patient. Myocarditis was excluded on the basis of the Dallas criteria[12] as well as the method of Edwards *et al*[13] and Katsuragi *et al*.[14] Immunohistochemical analysis with CD45RO was conducted to clarify T lymphocyte infiltration. In a quiescent condition with optimal medical treatment, patients underwent electrocardiography, echocardiography and blood sampling for standard laboratory chemical analysis, myocardial markers and complete blood count just before discharge from hospital. Myocardial markers measured in the present study were as follows: plasma BNP (Shionogi Co, Osaka, Japan), serum H-FABP (MARKIT-M, Dainippon Pharmaceutical Company, Osaka, Japan) and cardiac troponin T (cTnT) (Boehringer Mannheim,

Abbreviations: BNP, brain natriuretic peptide; cTnT, cardiac troponin T; DCM, dilated cardiomyopathy; H-FABP, heart-type fatty acid binding protein

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.

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.

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) ($p = 0.0068$ 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.

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 DCM. The predictive power of H-FABP was comparable to that of BNP.

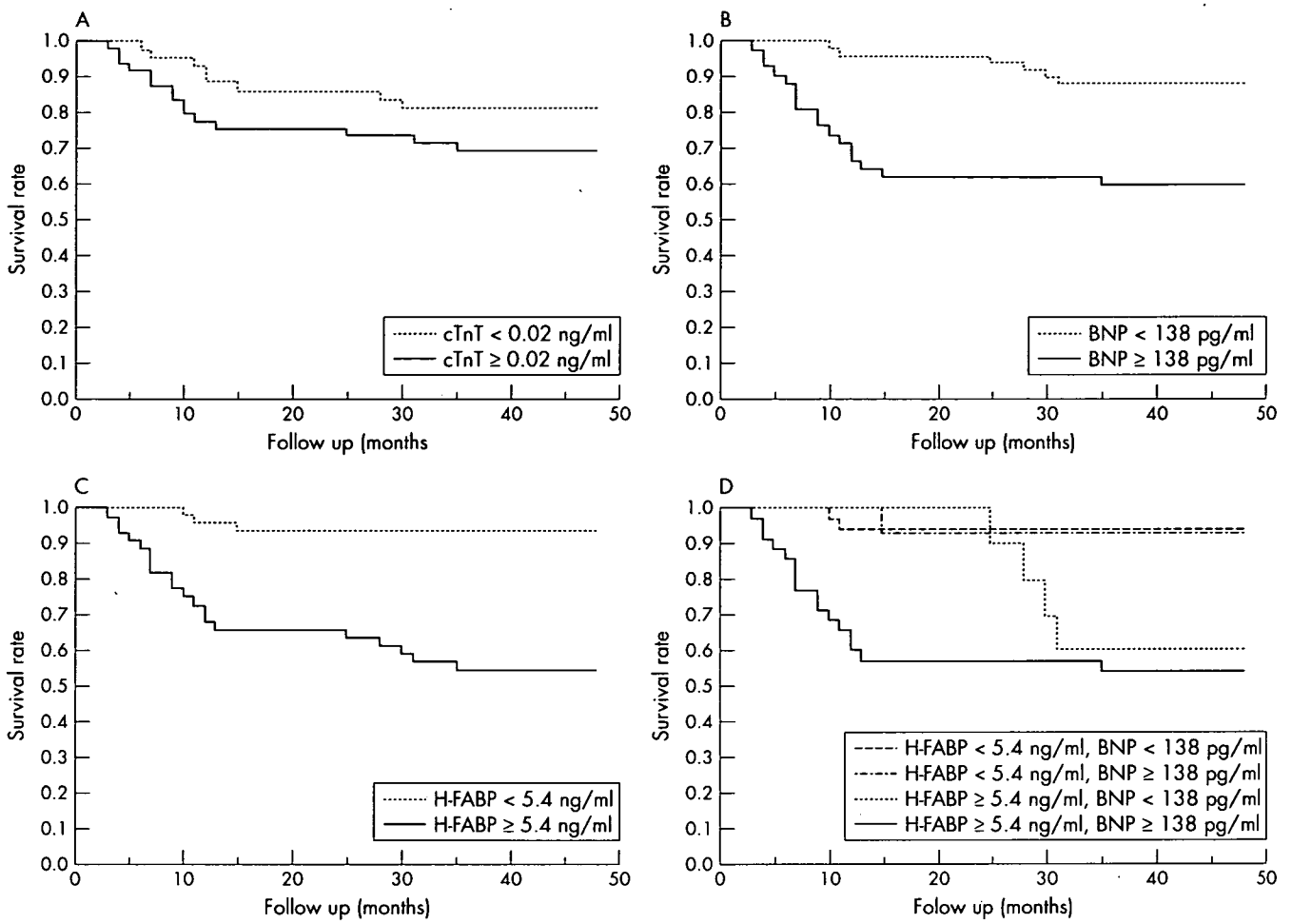

Figure 1 Kaplan–Meier event curves according to the median concentrations of (A) cardiac troponin T (cTnT), (B) brain natriuretic peptide (BNP), (C) heart-type fatty acid binding protein (H-FABP) and (D) H-FABP and BNP combined.

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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Prevention of Catheter-Related Infections Using A Closed Hub System in Patients With Pulmonary Arterial Hypertension

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Background Most of the patients with pulmonary arterial hypertension (PAH) receiving intravenous epoprostenol have experienced catheter-related infections during long-term treatment. Catheter hub was reported to be the most important source of catheter-related infections. To prevent the catheter-related infections, we have introduced a closed hub system and compared the incidence of catheter-related infections with that in patients using a non-closed hub system.

Methods and Results We evaluated the results obtained on 24 occasions in 20 patients with PAH between June 1999 and December 2005. On 11 occasions, a non-closed hub system was used and on 13 cases a closed hub system. We classified the catheter-related infection into a catheter-related bloodstream infection (CRBSI) group or a tunnel infection group based on the pathway of bacteria. The CRBSI rate was 0.89 per 1,000 catheter days in the non-closed hub system group vs 0.10 per 1,000 catheter days in the closed hub system group. Kaplan–Meier analysis showed that the risk of CRBSI significantly decreased in the closed hub system group. None of the patients died as a direct consequence of catheter-related infection during the study period.

Conclusions We successfully prevented CRBSI by using a closed hub system. (*Circ J* 2007; **71:** 559–564)

Key Words: Catheter-related bloodstream infection; Catheter-related infection; Closed hub system; Epoprostenol; Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a rare disease of poor prognosis. Recently, continuous intravenous epoprostenol (EPO) was introduced as a treatment for PAH with the consequent improvement of the patients' quality of life[1]. Although therapy with EPO improves the patients' exercise capacity and prognosis, various complications related to the central venous catheters arise during long-term treatment with EPO[2,3]. Among them, catheter-related infection is a serious problem, because the infections aggravate the primary disease.

The incidence of catheter-related infections was reported to be 0.3–9.1 infections per 1,000 patient-days in patients with long-term, indwelling central venous catheters for various diseases[4–6]. According to a recent report by Oudiz et al, at least 10% of their patients with a catheter infection required admission to critical care wards and several patients died as a direct consequence of the catheter infection, although the incidence of catheter-related infections in patients with PAH receiving EPO was lower than that in patients with other diseases[7]. Prevention of catheter-related infections would lead to further improvement of the prognosis of patients with PAH.

It has already been reported that the catheter hub was the most important source of catheter-related infections[8,9] and thus several closed hub systems have been introduced[10,11]. In the present study, we adopted the closed hub system for patients with PAH receiving EPO to prevent bacterial invasion from the catheter hub.

Methods

Closed Hub System

We introduced the closed hub system after March 2002. This system consists of 2 parts (Fig 1). One part is the Hickman catheter connected to an extension tube (Figs 1A, B,D), and the other part is the infusion port for EPO in medication baggage (Fig 1C). The connection between the Hickman catheter and extension tube is a commercially available catheter connection system (I-system; Nipro Corporation, Japan). The I-system consists of an I-plug (a cap with a latex end injection plug with a male screw) and an I-set (a 21-gauge needle with a Luer–Lock female screw) (Fig 1B). The I-plug is applied to the hub of the Hickman catheter (Fig 1A). The needle of the I-set is inserted into the latex end of the I-plug. Then, the I-plug and I-set are fixed together by the Luer–Lock. The infusion port for EPO in the medication baggage consists of a plug made of latex (Fig 1C).

Study Population

From June 1999 to December 2005, 20 patients with

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Fig 1. Closed hub system. (A) I-plug and Hickman catheter. (B) I-system consists of an I-plug and an I-set. (C) Infusion port for epoprostenol in medication baggage. (D) A whole picture of the closed hub system.

PAH receiving continuous therapy with EPO were referred to the National Hospital Organization Okayama Medical Center and to the Department of Cardiovascular Medicine of Okayama University. PAH was either idiopathic (19 patients) or caused by a connective tissue disease (1 patient) and was defined as PAH based on a mean pulmonary arterial pressure ≥25 mmHg at rest, pulmonary capillary wedge pressure ≤15 mmHg and a pulmonary vascular resistance of ≥240 dyne·s·cm^{-5}. All patients underwent an infusion of EPO via a central venous catheter (Hickman; CR Bard, Inc, NJ, USA). The trained physicians inserted the catheters into the subclavian vein in the angiography room. Before the insertion, the skin was disinfected with 10% povidone-iodine or chlorhexidine gluconate. The physicians performed a thorough hand scrub, wore sterile gloves, gown, cap, and mask, and used large sterile drapes. In 11 patients (4 men and 7 women) who were started on continuous therapy with EPO before August 2002, we used the non-closed hub system. And in 9 patients (2 men and 7 women) who were started on continuous therapy with EPO after January 2003, we used the closed hub system. In addition, in 4 patients (all women) who had suffered catheter-related infections with the non-closed hub system, this was changed to the closed hub system after March 2003. Patients were discharged from hospital after they were instructed on the care and maintenance of the catheter. All patients were monitored every 1–4 months. During the intervals, their private physicians were in the charge of the catheter care and maintenance. All patients were not treated with oral steroids. And there were no patients with immunodeficiency. The local institutional review boards approved the protocol of this study. All participating patients provided informed consent to participate in the study.

Care and Maintenance of the Catheter

The administration of EPO required a daily mixing of the drug in both systems. At first in the non-closed hub system, patients had to fill a backup infusion pump reservoir with EPO under sterile conditions. Then, the patients had to disconnect the catheter from the infusing pump and quickly connect it to the newly filled pump. Thus, the patients' extension tube connected to their central venous catheter, which was 'opened' every day.

In the closed hub system, the EPO was refilled from the infusion port in the medication baggage under sterile conditions. All the patients had to change the medication baggage and the extension tube every 3 days. Because the central venous catheter was closed by the I-plug, the catheter was never 'opened' while they were at home. A physician changed the I-plug using a sterile procedure when the patient visited the outpatient clinic every month. The tube and central venous catheter was reconnected using sterile procedures, without the use of a saline solution or heparin.

A sterile dry gauze or semi-permeable dressing was used to seal the catheter insertion site under sterile conditions every day. The skin around the catheter insertion site was disinfected with povidone-iodine or chlorhexidine gluconate at the time the dressing was changed. This dressing regimen was not changed over time. Physicians and nurses were instructed on the care and maintenance of the patient catheters. And patients were instructed to ask a nurse and/or a physician for an evaluation immediately if they noted erythema, edema, tenderness and/or a persistent discharge at the catheter insertion site or generalized low-grade fever.

Definitions of Catheter-Related Infections

When the patients suspected a catheter-related infection, they were instructed to immediately visit our hospital or a private physician. When the private physician diagnosed a catheter-related infection, he/she must have referred the patient to our hospital for admission or prescribed antibiotics. In fact, patients were admitted to our hospital when catheter-related infections did not improve after physician treatment. At the hospital, a chest radiograph was taken, and blood and urine examinations were carried out. We diagnosed a patient as having a catheter-related infection according to the 2002 CDC recommendations![12] Catheter-related bloodstream infections (CRBSI) was defined as at least 1 positive blood culture obtained from a peripheral vein, in a patient with clinical manifestations of infections (ie, fever, chills, and/or hypotension) and if there was no apparent source for bloodstream infection except for the catheter. Tunnel infection was defined as erythema, edema, tenderness, or drainage from the exit site. If necessary, we removed the Hickman catheter and cultured the catheter tip and pus from the insertion site.

Table 1 Patient Demographics

Variables	All patients (n=20)	Non-closed hub system (n=11)	Closed hub system (n=13)
Age, years	31.3±11.3	26.3±7.2	35.5±11.0
M/F	6/20	4/11	2/13
Diagnoses			
Idiopathic PAH	20	11	12
CTD	0	0	1
Catheter days	14,732	5,894	8,838

PAH, pulmonary arterial hypertension; CTD, connective tissue disease.

Table 2 Number of Catheter Infections in Patients With PAH Distributed According to the Type of System Used

Patient no.	Sex	Age (years)	System	Incidence of all catheter infection	Incidence of tunnel infection	Incidence of CRBSI
1	F	23	Non-closed	1	0	1
2	F	33	Non-closed	3	1	2
3	M	20	Non-closed	3	2	1
4	M	21	Non-closed	3	2	1
5	F	26	Non-closed	5	5	0
6	F	36	Non-closed	1	1	0
7	M	14	Non-closed	2	1	1
8	M	19	Non-closed	2	1	1
9	F	28	Non-closed	1	1	0
10	F	31	Non-closed	0	0	0
11	F	36	Non-closed	0	0	0
12	F	51	Closed	2	0	2
13	F	32	Closed	1	1	0
14	M	58	Closed	0	0	0
15	F	35	Closed	0	0	0
16	F	33	Closed	0	0	0
17	F	29	Closed	1	1	0
18	M	32	Closed	0	0	0
19	F	16	Closed	0	0	0
20	F	45	Closed	0	0	0
2*	F	33	Closed	0	0	0
5*	F	26	Closed	1	1	0
6*	F	36	Closed	0	0	0
9*	F	28	Closed	1	1	0

**Patients who were switched from the non-closed hub system.*
CRBSI, catheter-related bloodstream infection. Other abbreviation see in Table 1.

Table 3 Bacterial Species Isolated From Blood, Catheter Tip or Pus From the Insertion Site of PAH Patients

Organism	Instances of a non-closed hub system		Instances of a closed hub system	
	Tunnel infection	CRBSI	Tunnel infection	CRBSI
Methicillin-sensitive staphylococcus aureus	5	2	2	0
Micrococcus spp	1	1	0	1
Staphylococcus epidermidis	0	1	0	0
Coagulase negative staphylococci	1	0	0	0
Corynebacterium spp	0	0	1	0
Not cultured	4	3	1	1
Not collected	3	0	0	0

Figs indicate the number of times the microorganisms were isolated.
Abbreviations see in Tables 1, 2.

Statistical Analysis

Results are reported as the mean±standard deviation. Infection rates are reported as per 1,000 patient days. The cumulative risk of developing a catheter-related infection in the 2 groups as a function of the duration of catheterization was estimated according to the Kaplan–Meier method and compared with the use of the log rank test.

Results

The patient characteristics are shown in Table 1. The patients' age ranged from 15 to 58 years (mean age 31.3 years). The total number of catheter days was 5,894 days in the non-closed hub system group and 8,838 days in the closed hub system group. The duration of catheter indwelling was 281–1306 days, with an average of 536±367 days in the non-closed hub system group and 50–1,386 days, with an average of 680±370 days in the closed hub system group.

In the non-closed hub system group, 9 patients developed a total of 21 catheter-related infections during the study period (Table 2). Eight patients (14 catheter infections) had

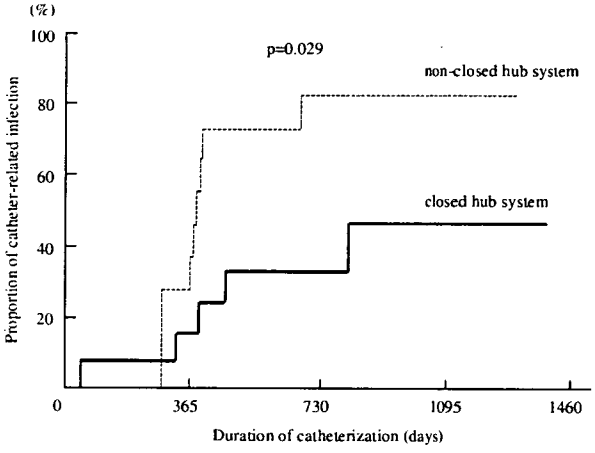

Fig 2. Analysis of the Kaplan–Meier estimates of the overall risk for catheter-related infections according to the duration of the catheterization.

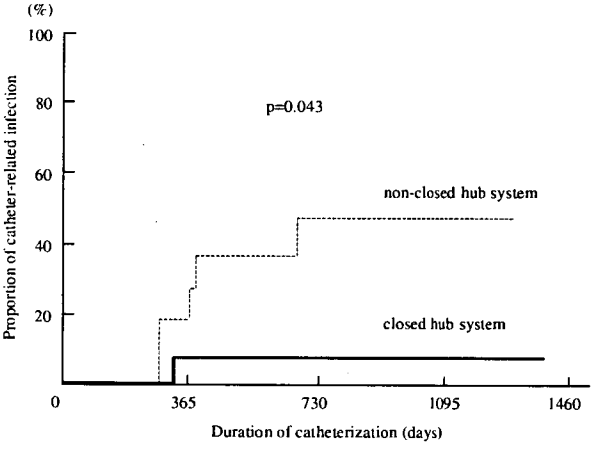

Fig 3. Analysis of the Kaplan–Meier estimates of the risk for catheter-related bloodstream infection according to the duration of the catheterization.

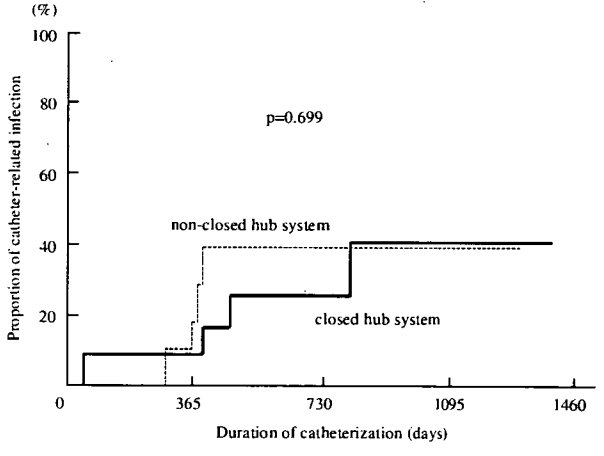

Fig 4. Analysis of the Kaplan–Meier estimates of the risk for tunnel infections according to the duration of the catheterization.

tunnel infections and 6 patients (7 catheter infections) had CRBSI. Five patients developed both types of infections.

In the closed hub system group, 5 patients developed a total of 6 catheter-related infections during the study period (Table 2). Four patients (4 catheter infections) had tunnel infections and 1 (2 catheter infections) had CRBSI. Two of the patients who developed tunnel infections had previously used the non-closed hub system, and one of them had suffered repeated tunnel infections (5 catheter infections) with the non-closed hub system.

All patients were admitted to either of our 2 centers. In the patients with tunnel infection, the catheter had to be removed as part of the treatment and they were administered antibiotics intravenously at least once a week, except for 1 patient. In 1 patient, treatment with just oral antibiotics was sufficient to eradicate the infection.

The etiologic organism was identified in at least one cultured specimen in 13 of the 27 clinical episodes of infections. The organisms isolated are listed in Table 3. Methicillin-sensitive staphylococcus aureus (MSSA) was the most frequent isolate in both groups. In the non-closed hub system, bacterial cultures were negative in 7 of the 21 clinical infections.

The overall catheter-related infection rate for our patients was 1.53 per 1,000 catheter days in the non-closed hub system group vs 0.45 per 1,000 catheter days in the closed hub system group. The CRBSI rate was 0.89 per 1,000 catheter days in the non-closed hub system group vs 0.10 per 1,000 catheter days in the closed hub system group. The tunnel infection rate was 0.87 per 1,000 catheter days in the non-closed hub system group vs 0.33 per 1,000 catheter days in the closed hub system group.

Kaplan–Meier estimates of the overall risk for catheter-related infections showed a significant difference between the non-closed hub system and the closed hub system (Fig 2, p=0.029). Furthermore, Kaplan–Meier estimates of the risk for CRBSI showed a significant difference between the non-closed hub system and the closed hub system (Fig 3, p=0.043). However, Kaplan–Meier estimates of the risk for tunnel infections showed no difference between the 2 groups (Fig 4, p=0.699).

None of the patients died as a direct consequence of a catheter-related infection during the study period.

Discussion

In the present study, the overall incidence of catheter-related infections and CRBSI was significantly decreased with the closed hub system. Furthermore, the incidence of CRBSI was extremely low in comparison to that found in a previous study[7]

Recently, the use of continuous infusion therapies at home, for example, total parenteral nutrition, has remarkably increased. This type of therapy has contributed not only to an increase in therapeutic options but also in terms of improving patients' quality of life. However, various problems associated with the management of this therapy still persist. Catheter-related infections, one of the most serious problems, can aggravate the primary disease and worse, might result in death. Catheter-related infections associated with the therapy have been evaluated with regard to various diseases.[13-16]

In the present study, catheter-related infections were divided into 2 groups according to the 2002 CDC recommendations. This difference can be explained by the path-

ways of bacterial invasion. CRBSI could be caused by bacterial invasion through the catheter connection. Tunnel infection could be caused by direct bacterial invasion through the catheter insertion site. To prevent catheter-related infections, we need to identify which pathway of bacterial invasion was predominant in an individual case.

Previously, it was considered that direct bacterial invasion through the catheter insertion site was the main cause of catheter-related infections[17,18] To prevent direct bacterial invasion, it was thought that the catheter insertion site had to be cleaned and the subcutaneous tunnel had to be long. However, it was reported that the results of bacterial culture from the catheter insertion site did not necessarily coincide with those of blood and catheter tip cultures[19,20] In addition, the use of long subcutaneous tunnels failed to decrease the frequency of catheter-related infections[21,22] As a result, attention has now focused on bacterial invasion through the catheter connection. Sitges-Serra et al evaluated the possibility of bacterial contamination at the catheter connection and postulated the "hub hypothesis"[8] They reported that the hub of a catheter was the most important source of catheter-related infections[9] This hypothesis has been supported by several studies[23–26] Consequently, we adopted the closed hub system for the catheter connection in our patients. The system we used simplifies catheter care and infusion line use compared with the non-closed hub system. Bacterial contamination can be minimized with this system because the catheter connection is not open to air during the change of the infusion line except at the monthly change of the I-plug in the clinic. The effectiveness of this system in reducing CRBSI was already reported in patients with other diseases[10] In the present study, we showed that we could significantly decrease CRBSI by using the closed hub system in patients with PAH receiving EPO.

As for microorganisms causing catheter infections, we found that MSSA was the most frequent cause of infection, which is in agreement with the results of a previous study involving patients with PAH[7] In that study, micrococcus spp was the second most common etiologic agent. The clinical syndrome of micrococcal catheter infection presented as generalized weakness and fatigue with or without fever[7] Although this symptom was common in the case of CRBSI with a non-closed hub system, cultures were positive in 1 patient. This could be because of micrococcus spp might have been present; this was ignored as a possible cause in our institute until 2003[27]

Although we could decrease the overall occurrence of catheter-related infections by reducing CRBSI, approximately 50% of patients still experienced catheter-related infections during 3 years. Most of the catheter-related infections were of the tunnel type and thus it is important to focus on the prevention of tunnel infections in the future.

When a patient develops a catheter-related infection, the clinician must treat it as early as possible. However, early detection of catheter-related infections is difficult. Thus, when a patient receiving EPO starts to complain of constitutional symptoms for no apparent reason, clinicians should suspect the possibility of catheter-related infections and carry out a thorough investigation to rule out this possibility.

This study has several limitations. This is an observational and not a prospective study. We might have underestimated the true incidence of catheter-related infections. If the catheter infection was slight, clinicians might have been unaware of it. Clinicians treat catheter-related infections with antibiotics based on the diagnosis of other infections.

Conclusions

The present study demonstrated that the use of the closed hub system reduced the overall occurrence of catheter-related infections in patients with PAH receiving continuous therapy with EPO at home. Furthermore, the risk of CRBSI was significantly decreased with this system. Besides, the incidence of CRBSI was low compared with another study involving patients with PAH receiving continuous therapy with EPO.

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Highly Concentrated Vascular Endothelial Growth Factor in Crow-Fukase Syndrome With High Output Heart Failure: A Case Report

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Abstract

Crow-Fukase syndrome is a disease of plasma cell dyscrasia. Congestive heart failure is the biggest complication affecting the prognosis. A 57-year-old male was admitted with edema and low grade fever. Globe and stocking type polyneuropathy, increased levels of adrenocorticotropic hormone and thyroid-stimulating hormone, serum M-protein component of the immunoglobulin A-λ type, skin polypoid lesion, and organomegaly including cardiomegaly were observed. The diagnosis was Crow-Fukase syndrome based on these clinical features. High output heart failure and pulmonary hypertension were determined with a cardiac catheter. Diuretics and angiotensin converting enzyme inhibitor were effective to control his overhydration. The level of serum vascular endothelial growth factor was markedly increased and might be responsible for the manifestation of this syndrome with cardiac involvement.

J Cardiol 2005 Feb; 45(2): 75-80

Key Words

■ **Heart failure (high cardiac output)** ■ **Hypertension, pulmonary** ■ **Complications**
■ **Endothelium (vascular endothelial growth factor)**

INTRODUCTION

Crow-Fukase syndrome is a disease of plasma cell dyscrasia which is associated with polyneuropathy, endocrinopathy, M-protein, skin lesions and organomegaly[1]. Congestive heart failure is the most important factor affecting the prognosis[2]. Although the etiology of Crow-Fukase syndrome is not known, recent reports have suggested that serum vascular endothelial growth factor (VEGF) plays an important role[3-6]. We report a case of Crow-Fukase syndrome with high output heart failure and pulmonary hypertension associated with serum VEGF elevation.

CASE REPORT

A 57-year-old male was admitted to our hospital for further examination of cardiomegaly on February 26, 1996. He had suffered foot edema, numbness of lower extremities and low grade fever for 6 months before admission. The following physical factors were recorded at the time of admission: height, 160 cm; body weight, 62.4 kg; body temperature, 37.2 ℃; blood pressure, 160/74 mmHg; and pulse rate, regular at 92 beats/min. Physical examination revealed diffuse skin pigmentation

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with polypoid skin tumor and pitting edema of the lower extremities. Clubbing was not detected. The jugular vein was dilated. A systolic murmur was audible at the third intercostal space near the left sternal border (Levine Ⅲ/Ⅵ). No lung rales were detected. The abdomen was distended and hepatosplenomegaly was detected. Neurological examination revealed diminished bilateral patella and Achilles tendon reflex. Complete blood cell count showed normocytic normochromic anemia (erythrocyte count = $332 \times 10^4/\mu l$, hemoglobin value = 10.1 g/d*l*, hematocrit value = 30.5%) with normal white cell and platelet count. Blood chemistry revealed mild hypocholesterolemia (total cholesterol = 121 mg/d*l*), slightly decreased cholinesterase (125 IU/*l*) and mild elevation of blood urea nitrogen (25.1 mg/d*l*). C-reactive protein level was 1.0 mg/d*l*. Serum immunoglobulin A level was elevated (735 mg/d*l*) and serum immunoelectrophoresis revealed an M-protein component of the immunoglobulin A-λ type without urine Bence Jones protein. Adrenocorticotropic hormone was elevated (82 pg/m*l*) with normal cortisol level and free T_3 level was slightly decreased (1.75 pg/d*l*) with mild thyroid-stimulating hormone elevation (4.0 μIU/m*l*). Serum thiamine level was slightly low (17 ng/m*l*) and mild elevation of serum interleukin-6 was observed (4.3 pg/m*l*). Renin activity, aldosterone and catecholamines (adrenaline, noradrenaline and dopamine) in the plasma were all normal.

Chest radiography revealed cardiomegaly (cardiothoracic ratio = 56%) with mild pulmonary congestion (**Fig. 1**). Electrocardiography showed R wave retraction in leads V_3-V_4, biphasic T wave in leads V_2-V_5 and ST segment depression in leads I and V_5-V_6 (**Fig. 2**). Abdominal computed tomography showed hepatosplenomegaly with mild ascites. The conduction velocities of the median nerve and tibian nerve were decreased. Bone scintigraphy showed no osteolytic or osteoclastic lesion. Bone marrow puncture showed mild plasmacytosis without plasmacytoma. Lumbar puncture was not performed. Echocardiography showed left ventricular hypertrophy and dilation with normal contractility (interventricular septal thickness, left ventricular posterior wall thickness, left ventricular end-diastolic diameter, left ventricular end-systolic diameter and ejection fraction were 14, 12, 65, 40 mm and 68% respectively). The left atrium and aortic root were slightly dilated and mild pericardial effusion was detected (left atrial diameter and aortic diameter were 38 and 40 mm; **Fig. 3**). Doppler echocardiography showed mild aortic valve stenosis (pressure gradient = 20 mmHg) and moderate tricuspid regurgitation (estimated right ventricular pressure = 52 mmHg). Based on these signs and laboratory data, the diagnosis was Crow-Fukase syndrome.

Fig. 1 Chest radiograph on admission

Diuretic administration (furosemide, 40 mg/day) was started for the treatment of edema and ascites. Stress thallium myocardial scintigraphy showed no perfusion defect. Right heart catheterization performed on 26th day under no medication revealed pulmonary hypertension and high cardiac output with low systemic and pulmonary vascular resistance (**Table 1**). Oxygen saturation evaluated at the cardiac catheterization did not show any significant shunt between the right and left heart (**Table 2**). Selective coronary angiography was normal and left ventriculography showed left ventricular dilation with normal contractility. Histological examination of the biopsy specimen obtained from the left ventricle showed mild myocardial hypertrophy with disarray. The diagnosis was high output heart failure with pulmonary hypertension.

In addition to the treatment with diuretics for overhydration, hypertension with left ventricular hypertrophy was treated with calcium antagonist

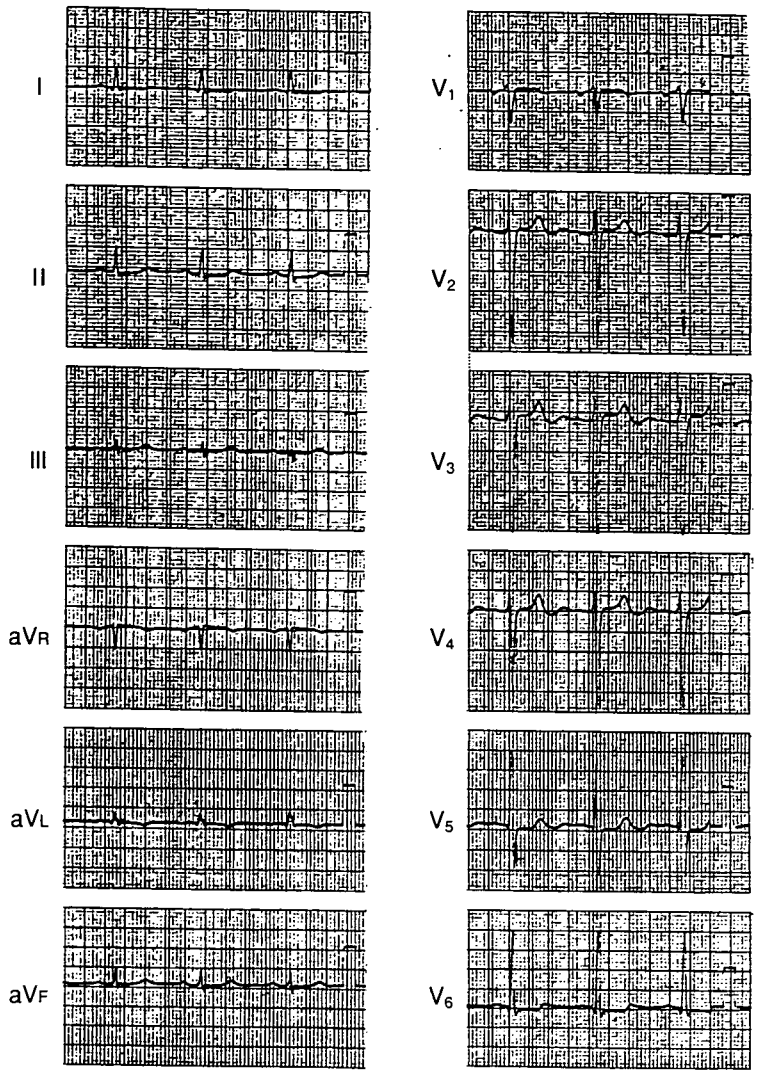

Fig. 2 Electrocardiogram on admission

(amlodipine, 5 mg/day) and angiotensin converting enzyme inhibitor (derapril, 30 mg/day). In the absence of severe neurological deficit, no steroid hormone was used. He was discharged after the improvement of pulmonary congestion, ascites and pitting edema. Blood urea nitrogen, creatinine and serum potassium ion levels increased, furosemide level was decreased (40 → 20 mg/day) and deraplil medication was stopped.

His clinical signs and laboratory data have not changed for 7 years during follow up in the outpatient clinic. Organomegaly has persisted as determined by abdominal computed tomography and echocardiography. However, serial echocardiography showed diminished left ventricular volume and right ventricular systolic pressure (left ventricular end-diastolic diameter, end-systolic diameter and estimated right ventricular systolic pressures were 58 mm, 38 mm and 45 mmHg on May 19, 1999; 52 mm, 35 mm and 26 mmHg on June 11, 2003). Marked elevation of vascular endothelial growth factor (1,280 pg/m*l*) and slight elevation of hepatocyte growth factor (0.42 pg/m*l*) were observed during follow up at the outpatient clinic on June 11, 2003. He was readmitted to our hospital for the increase of ascites despite treatment with diuretics on January 16, 2004. Osteosclerotic lesion was found in his left iliac bone for the first time. He was referred to the division of hematology of another hospital for treatment with melphalan and prednisolone.

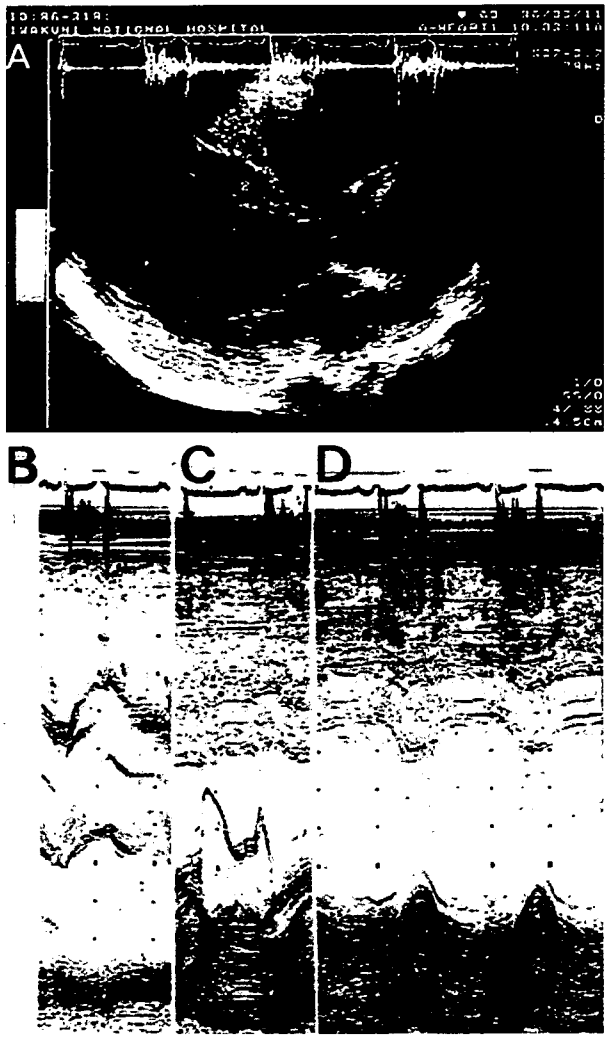

Fig. 3 Echocardiograms on admission
A: Parasternal long-axis B-mode view in enddiastole.
B: M-mode view of the right ventricle, aorta and left atrium.
C: M-mode view of the mitral valve.
D: M-mode view of the left ventricle at the level of the chordae tendineae.

Table 1 Hemodynamic findings

Pulmonary capillary wedge pressure	14 mmHg
Pulmonary artery pressure	43/17 mmHg
Right ventricular pressure (EDP)	42/5 (7) mmHg
Right atrial pressure	5 mmHg
Left ventricular pressure (EDP)	182/0 (13) mmHg
Aortic pressure	160/74 mmHg
Cardiac output	11.2 *l*/min
Cardiac index	6.63 *l*/min/m^2
Stroke volume	183.0 m*l*/beat
Stroke index	108.3 m*l*/beat/m^2
Total vascular resistance	727.8 dyn·sec·cm^{-5}
Total pulmonary resistance	192.5 dyn·sec·cm^{-5}
Heart rate	62 beats/min
Body surface area	1.69 m^2

EDP=end-diastolic pressure.

Table 2 Oxygen saturation at cardiac catheterization

Site	Saturation (%)
Superior vena cava	88.0
Inferior vena cava	88.6
Right atrium	80.0
Right ventricle	85.5
Proximal pulmonary artery	85.0
Distal pulmonary artery	84.4
Left ventricle	98.2
Aorta	98.2

Samples were obtained from the patient breathing room air.

DISCUSSION

We did not follow the recommended strategy using alkylators with or without steroid hormone in the present patient because: clubbing, a sign associated with shorter survival, was not detected and extra vascular volume overload was controllable with diuretics[7]; neurological deficit was mild and gait disturbance was not detected; the prognosis was reported to be better in a recent review[7] than in former cases[2] (median survival 165 months vs 33 months); and VEGF, a useful marker associated with the effect of treatment[3-6], could not be measured at the first admission without a commercially available kit. The use of vasodilating agents for the lowering of the blood pressure may have increased cardiac output. However, loop diuretics may have canceled the adverse effect of the vasodilating agents and controlled the signs of overhydration without additional use of thiazide or spironolactone. However, we planned chemotherapy for the osteosclerotic lesion and refractory ascites despite reduced cardiac output and improved cardiomegaly with diuretic treatment after 7 years follow-up.

The cause of one third of deaths in patients with this syndrome is congestive heart failure[2]. On the other hand, the elevation of serum VEGF probably causes the symptoms of this syndrome[3-6]. Only a few reports have evaluated the relationship between VEGF and hemodynamic disorder of this syndrome. A case with serum VEGF elevation and