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Creatine Kinase Isoforms as Circulating Markers of Deterioration in Idiopathic Dilated Cardiomyopathy

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Summary

Background: A proportion of patients with dilated cardiomyopathy (DCM) may have ongoing myocardial damage secondary to viral or immune mediated myocardial inflammation.

Hypothesis: The prognostic determinants identify patients with decreased survival but do not provide a measure of myocardial damage. To obtain an objective assessment of myocardial damage in DCM, we measured plasma levels of creatine kinase (CK), its isoenzymes (CK-MM and CK-MB), and separated the isoforms of CK-MM and CK-MB.

Methods: The cohort consisted of 77 consecutive patients (61 men, 16 women) with DCM (World Health Organization criteria), aged 49 ± 14 years (range 19–60). Patients had been symptomatic for 29 ± 38 months (range 0.5–200 months) with 48 in New York Heart Association class I/II and 29 in class III/IV at the time of diagnosis. During median follow-up of 27 months from diagnosis (range 0.6–165), 50 patients remained clinically stable and 27 had deteriorated.

Results: A significantly higher proportion of patients with DCM had abnormal MB2/MB1 ratio compared with normal volunteers (11, 14% vs. 1, 1%, p = 0.003). Patients who deteriorated had higher MB2/MB1 ratio, (1.22 ± 0.62 vs. 0.85 ± 0.56; p = 0.01), and more frequently had abnormal MB2/MB1 ratio (8, 30% vs. 3, 6%; p = 0.004) and CK and CK-MM activities (5, 19% vs. 2, 4%; p = 0.03) than those who remained stable. Patients with DCM with high CK-MB activity had 3.13-fold increased odds of sudden death or need for cardiac transplantation (95% confidence interval 1.53–6.40, p = 0.008). Thus, CK measurements, in particular CK-MB isoforms, are markers of myocardial damage in a subset of patients with DCM and could be useful in investigating the possibility of persistent myocardial damage in these patients.

Key words: dilated cardiomyopathy, cardiac enzymes, myocardial damage

Introduction

Dilated cardiomyopathy (DCM) is a chronic heart muscle disease of unknown etiology and poor prognosis[1,2] characterized by a dilated and poorly contractile left ventricle.[3] In a proportion of patients there is chronic myocardial damage which may relate to persistent viral infection[4] or immune mediated damage.[5-8]

Several prognostic determinants have been identified in DCM, including functional status, left ventricular (LV) function, exercise capacity, ventricular arrhythmic activity, and neurohumoral activation.[9-14] These functional indices identify patients with decreased survival but do not provide a measure of ongoing myocardial damage and have provided no practical guidelines for the management of patients with DCM.[15]

Elevated levels of circulating creatine kinase (CK) MM isoforms (MM3: tissue isoform, MM1: plasma-modified isoform) have been found in patients with hypertrophic cardiomyopathy (HCM),[16] suggesting myocardial damage in these patients, and have also been used for diagnosis of patients with acute myocardial infarction (AMI),[17] but these isoforms are not cardiac-specific.[18] The two isoforms of CK-MB (MB2: the tissue, and MB1: the plasma-modified isoform) are more specific for myocardial injury. They have been used as sensitive markers of ischemic myocardial damage as well as for early diagnosis of AMI.[19,20]

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Creatine kinase isoforms have not been previously assessed in DCM. The aim of this study was to evaluate circulating CK isoforms as markers of myocardial damage in a consecutive cohort of patients with DCM and to determine their value as prognostic determinants.

Methods

Patients with Dilated Cardiomyopathy

The study population consisted of 77 consecutive patients (61 men, 16 women) with DCM (mean age 49 ± 14 years, range 19–60) who presented to our hospital between 1989 and 1993. The clinical diagnosis of DCM was made according to strict criteria as recommended by the World Health Organization and the National Heart, Lung, and Blood Institute.[3,21] All patients had LV dilatation (end-diastolic diameter > 2.7 cm/m^2) and impaired systolic contraction (LV ejection fraction <40% or fractional shortening < 25%). Exclusion criteria included ≥ 50% obstruction of one or more coronary arteries, active myocarditis,[22] specific primary or secondary heart muscle disease, isolated right ventricular dilatation, valvular or pericardial disease, systemic hypertension, or excess alcohol consumption (men: >21 units per week; women: >14 units per week) where ventricular dilatation persisted despite abstinence from alcohol.

Normal Volunteers

We studied 76 normal volunteers (70 men, 6 women) with no evidence of cardiac or other disease (mean age 29 ± 8 years, range 19–60).

Definitions

Sudden death was defined as death within 1 h of the onset of new symptoms.[23] The definition included instantaneous death, death during sleep, as well as unwitnessed death that occurred within 1 h of the patient last being seen alive. Progressive heart failure was defined as deteriorating symptoms of congestive heart failure despite maximal medical therapy and with increasing ventricular diameter at echocardiography. The predicted LV diastolic diameter for each patient was calculated according to a standard formula.[24] The predicted oxygen uptake at peak exercise was calculated on the basis of age and body surface area.[25] Sustained ventricular tachycardia was defined as spontaneous symptomatic ventricular tachycardia with a rate of > 120 beats/min, lasting for at least 30 s; nonsustained ventricular tachycardia was defined as spontaneous symptomatic ventricular tachycardia with a rate of > 120 beats/min, consisting of three or more consecutive ventricular ectopic beats and persisting for < 30 s.

Our hospital's Ethics Committee gave approval for this study, and informed consent, where appropriate, was obtained from each patient.

Clinical Assessment

Patients were assessed noninvasively by two-dimensional transthoracic Doppler echocardiography, 24-h ambulatory electrocardiographic (ECG) monitoring, radionuclide ventriculography, and maximal symptom-limited exercise testing with continuous monitoring of gas exchange (VO_2 max.). Left ventricular cavity dimensions were assessed using M-mode-guided short-axis views at the level of the papillary muscles. Measurements were made by a single experienced echocardiographer; all echocardiograms were reviewed by a second independent interpreter.

Invasive assessment consisted of cardiac catheterization with left ventriculography and right ventricular endomyocardial biopsy. Selective coronary angiography and biopsy was performed in all patients >30 years of age and in those patients < 30 years of age who had symptoms of chest pain or ischemic changes on exercise ECG; hemodynamic evaluation included evaluation of LV end-diastolic pressure and pulmonary capillary wedge pressure. If coronary angiography was not indicated, right ventricular biopsy alone was performed in those patients in whom active myocarditis was suspected clinically (symptoms and/or ECG changes). Histological assessment was performed by an experienced histopathologist using the Dallas criteria.[22]

Follow-Up

All patients were followed at a specialized heart failure clinic for a median duration of 27 months (range 0.6–165). At the end of the follow-up period, patients were categorized as stable (n = 50) or deteriorated [sudden death (n = 4) or development of progressive heart failure (n = 23), of whom 14 required transplantation].

Procedures for Blood Collection and Separation

Blood samples (5 ml) were collected into tubes containing disodium ethyl diamene tetra acetic acid (EDTA), at a final concentration of 15 mmol/l. This concentration of EDTA inhibits carboxypeptidase N-mediated isoform conversion after sample collection.[26] The blood samples were then centrifuged for 10 min at 2000 rpm, and the plasma removed for storage at −20°C until enzyme analysis.

Analytical Assays

Plasma total CK activity was measured using CK NAC reagent (Roche Diagnostic Systems, Nutley, N.J.) at 37°C, in a Cobas Mira auto analyzer. Plasma total CK-MB activity was measured immunochemically using Roche's Isomune kit based on the methodology of Wicks *et al.*[27] and was quantified using CK NAC reagent (Roche).

The CK-MM isoenzyme and each of its isoform fractions MM3, MM2, MM1, together with the MB2 and MB1 isoforms were separated by high-resolution agarose gel electrophoresis, and the percentages were quantified by densito-

metric scanning (REP, Helena Laboratories UK Ltd., Tyne & Wear, U.K.).[28, 29] The activity of the CK-MM isoenzyme was calculated by multiplying the percentage in the fraction, determined by electrophoresis, by the total CK activity. The MB2/MB1 and MM3/MM1 ratios were calculated by dividing the percentage of MB2 and MM3 in the fractions, derived from densitometry, by those of MB1 and MM1, respectively.

Statistical Analysis

The clinical and biochemical data are presented as mean ± standard deviation (SD). Between-group comparisons were performed using Kruskal-Wallis one-way analysis of variance, with pair-wise comparison using a Mann-Whitney U test. Between-group comparisons of patients with abnormal results were performed using chi^2 test. Spearman's correlation coefficient was used to correlate variables. The odds (relative risk) ratios of clinical and biochemical parameters for sudden death or transplantation were calculated based on the upper 10 percentile of values in the stable group as the definition of a raised value. The New York Heart Association (NYHA) functional classes I and II were taken as normal, and III and IV as abnormal. The p values and 95% confidence intervals were also calculated. A probability value of <0.05 was considered to be statistically significant.

Results

Clinical Parameters in Patients with DCM

Clinical assessment revealed that 48 patients were in NYHA functional classes I and II, and 29 patients were in class III or IV at the time of diagnosis. Patients had been symptomatic for 29 ± 38 months (range 0.5–200). Fifty-nine patients (78%) were in sinus rhythm and 14 (18%) in established atrial fibrillation; 23 (31%) had left bundle-branch block. Thirty (59%) patients had nonsustained ventricular tachycardia on Holter monitoring, and 52 (68%) patients had radiological evidence of pulmonary venous hypertension. Echocardiography demonstrated a mean LV diastolic diameter of 69 ± 10 mm (mean LV diastolic diameter was 141 ± 22% of predicted value), and a fractional shortening of 14 ± 7%. Patients had a mean angiographic ejection fraction of 26 ± 10%. Mean pulmonary capillary wedge pressure was 20 ± 9 mm/Hg, and the LV end-diastolic pressure was 20 ± 9 mm/Hg. Of 51 patients who underwent endomyocardial biopsy, 32 (63%) had established fibrosis, 1 (2%) had histologic evidence of active myocarditis, and 1 (2%) had evidence of resolving myocarditis; 18 (35%) biopsies were normal. Maximal exercise testing revealed a mean VO_2 max. of 23 ± 11 ml/min/kg (mean VO_2 was 25 ± 62% of predicted value).

Creatine Kinase Measurements in Normal Volunteers and Patients with Dilated Cardiomyopathy

The mean (± SD) and the percentage number of subjects with abnormal values for all CK measurements in 76 normal volunteers and 77 patients with DCM are shown in Table I. The upper limit of normal (ULN), defined as mean ± 2 SD, based on the in-house results, for CK, CK-MM, and CK-MB activities, MB2/MB1 and MM3/MM1 ratios were 309U/l, 296U/l, 9.6U/l, 0.5 and 1.5, respectively.

TABLE I Creatine kinase measurements in normal volunteers and patients with dilated cardiomyopathy (DCM)

	Normal volunteers (n = 76)	Patients with DCM (n = 77)	p Value
CK (U/l)	110 ± 93	144 ± 113	0.02
AbN CK; n (%)	3 (4)	7 (9)	NS
CK-MM (U/l)	100 ± 91	136 ± 110	0.001
AbN CK-MM; n (%)	3 (4)	7 (9)	NS
CK-MB (U/l)	3.9 ± 2.8	5.6 ± 5.4	0.001
AbN CK-MB; n (%)	3 (4)	7 (9)	NS
MM3/MM1	0.19 ± 0.15	0.23 ± 0.18	NS
AbN ratio MM3/MM1; n (%)	3 (4)	5 (7)	NS
MB2/MB1	0.63 ± 0.42	0.98 ± 0.61	0.0001
AbN ratio MB2/MB1; n (%)	1 (1)	11 (14)	0.003

Abbreviations: CK = creatine kinase, AbN = abnormal, NS = not significant.

A significantly higher proportion of patients with DCM had abnormal MB2/MB1 ratio compared with normal volunteers (11, 14% vs. 1, 1%, p = 0.003) (Table I). A scattergram of MB2/MB1 ratio in normal volunteers and patients with DCM is shown in Figure 1. However, for all the other CK measurements, the proportions of subjects with abnormal values were similar in both groups with no significant differences between them. All CK measurements in the patients with DCM were significantly higher than those in normal volunteers, with the exception of MM3/MM1 ratio (Table I, Fig. 2).

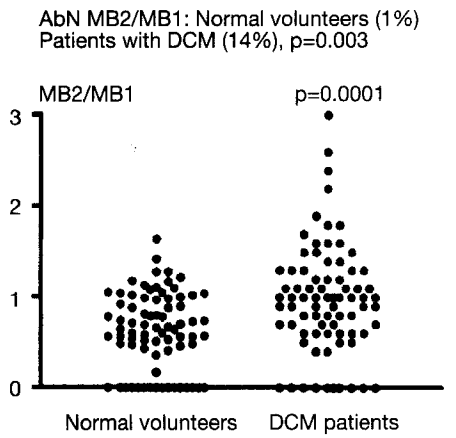

FIG. 1 Scattergram of MB2/MB1 ratio in normal volunteers and in patients with dilated cardiomyopathy (DCM).

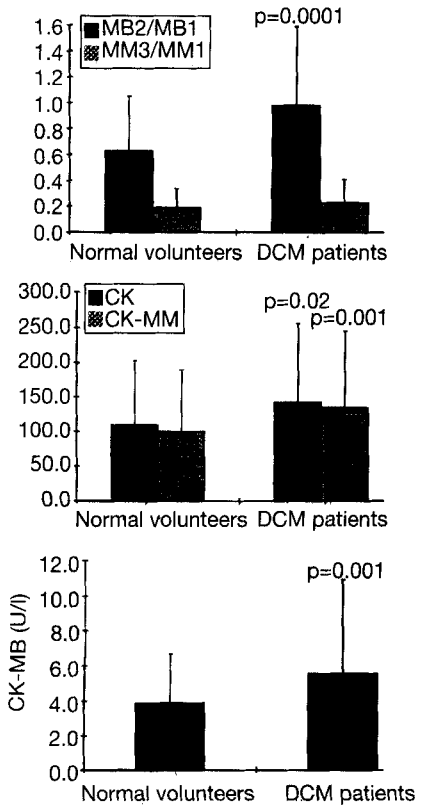

FIG. 2 Creatine kinase (CK) measurements in normal volunteers and in patients with dilated cardiomyopathy (DCM).

TABLE II Relationship between creatine kinase measurements and prognosis in patients with dilated cardiomyopathy

	Stable (n = 50)	SD or PHF (n = 27)	p Value
CK (U/l)	124 ± 72	177 ± 155	NS
AbN CK; n (%)	2 (4)	5 (19)	0.03
CK-MM (U/l)	117 ± 71	169 ± 153	NS
AbN CK-MM; n (%)	2 (4)	5 (19)	0.03
CK-MB (U/l)	4.6 ± 2.7	7.3 ± 8.0	NS
AbN CK-MB; n (%)	3 (6)	4 (15)	NS
MM3/MM1	0.22 ± 0.18	0.23 ± 0.18	NS
AbN ratio MM3/MM1; n (%)	3 (6)	2 (7)	NS
MB2/MB1	0.85 ± 0.56	1.22 ± 0.62	0.01
AbN ratio MB2/MB1; n (%)	3 (6)	8 (30)	0.004

Abbreviations: CK = creatine kinase, AbN = abnormal, SD = sudden death, PHF = progressive heart failure, NS = not significant.

Clinical and Biochemical Parameters and Outcome

The 27 patients who deteriorated had worse symptoms (NYHA I:II/III:IV 12/15 vs. 36/14; p = 0.006), larger LV diastolic diameter (74 ± 10 mm vs. 65 ± 8 mm; p = 0.0003), lower ejection fraction (19 ± 10% vs. 28 ± 10%; p = 0.01), and worse oxygen consumption at peak exercise (18 ± 7 ml/min/kg vs. 25 ± 10 ml/min/kg, p = 0.03) than those who remained stable.

The relationship between CK measurements and clinical outcome is shown in Table II and Figure 3. A greater proportion of patients who deteriorated had abnormal MB2/MB1 ratio compared with stable patients (8, 30% vs. 3, 6%; p = 0.004), and abnormal CK and CK-MM activities (5, 19% vs. 2, 4%, p = 0.03). The mean MB2/MB1 ratio was also significantly higher in patients who deteriorated than in those who remained stable (1.22 ± 0.62 vs. 0.85 ± 0.56; p = 0.01) (Fig. 4).

The MB2/MB1 ratio was associated with LV end-diastolic diameter (r = 0.23, p = 0.008), but there were no significant associations between the other CK measurements and clinical parameters including age, gender, functional class, ventricular diameters, ventricular contractility, or oxygen uptake at peak exercise. There was no association between MB2/MB1 ratio and histologic status.

Relative Risk of Deterioration

The odds (relative risk) ratios of the clinical and biochemical parameters for sudden death or transplantation were calculated (Table III). Of all parameters, only elevated CK-MB activity and NYHA functional class were predictive (p = 0.008, p = 0.03, respectively) in identifying patients at risk of death or transplantation.

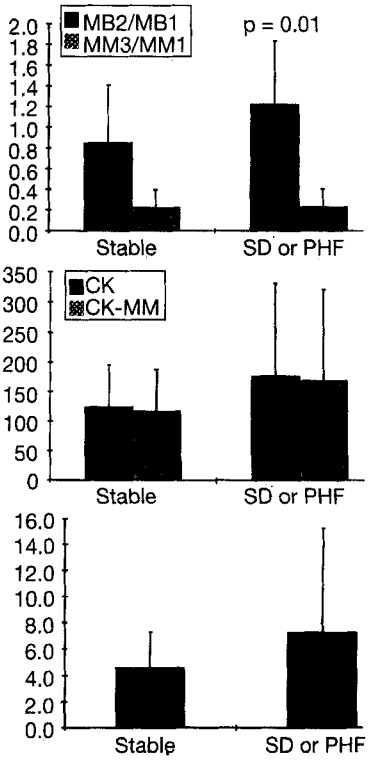

FIG. 3 Relationship between creatine kinase (CK) measurements and prognosis in patients with dilated cardiomyopathy. SD = sudden death, PHF = progressive heart failure.

TABLE III The odds (relative risk) ratios of sudden death or transplantation for the clinical and biochemical parameters in patients with dilated cardiomyopathy

Parameters	Odds (relative risk) ratios	95% Confidence intervals	p Value
NYHA	2.60	1.16–5.81	0.03
LVDD (mm)	2.29	0.98–5.32	NS
EF (%)	0.47	0.03–6.98	NS
FS (%)	0.38	0.03–5.47	NS
VO_2 max (ml/min/kg)	0.47	0.03–6.56	NS
CK	1.96	0.86–4.46	NS
CK-MM	1.61	0.65–3.99	NS
CK-MB	3.13	1.53–6.40	0.008
MM3/MM1	1.36	0.48–3.87	NS
MB2/MB1	1.93	0.88–4.22	NS

Abbreviations: NYHA = New York Heart Association, LVDD = left ventricular diastolic diameter, EF = ejection fraction, FS = fractional shortening, CK = creatine kinase, NS = not significant.

Discussion

Creatine kinase-MB isoforms have been shown to be sensitive markers of myocardial injury,[19, 20, 30] but have not been previously evaluated in DCM, although acute myocarditis is associated with abnormal CK levels.[31] To our knowledge, there are no data in the literature as to the effect of age, height, or body weight on CK isoform levels. In the present study, the cardiac-specific MB2/MB1 ratio was abnormal in 14% of patients with DCM compared with only 1% in the normal volunteers. Conversely, the proportions of subjects with abnormal values of all the other CK measurements were similar in both the patients with DCM and normal volunteers. Abnormal levels of CK-MM isoforms have been reported in patients with polymyositis,[18] but were uncommon in the present study (9%). The mean MM3/MM1 ratio was also found not to be significantly different between patients with DCM and normal volunteers. However, the mean values for all other CK measurements were found to be significantly higher in the patients with DCM than in normal volunteers.

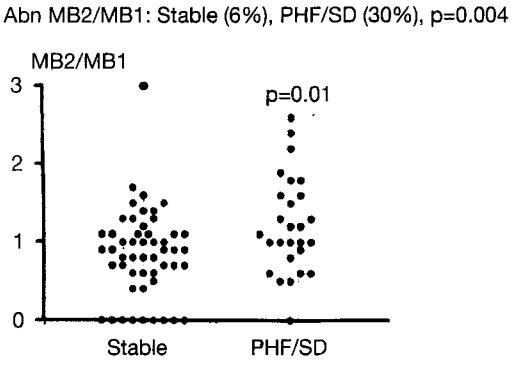

FIG. 4 Scattergram of MB2/MB1 ratio in patients with dilated cardiomyopathy with stable disease versus those with progressive disease. PHF = progressive heart failure, SD = sudden death.

A greater proportion of patients with adverse clinical outcome had abnormal MB2/MB1 ratio (30%) compared with those who remained clinically stable (6%), and the MB2/MB1 ratio was significantly higher in patients who deteriorated compared with those who remained stable, suggesting that there may be an association between release of CK-MB isoforms and adverse clinical outcome. A similar association between abnormal CK and CK-MM activities and adverse prognosis was also identified. Previous reports have evaluated a number of prognostic determinants for identification of patients at risk of progressive heart failure. Clinical status, angiographic ejection fraction, and LV diastolic diameter are the most important predictors of deterioration.[11] The present study examined only a small number of patients, but found that elevated CK-MB activity conferred a greater risk of adverse prognosis than the existing prognostic variables.

The potential causes of CK release from the myocardium include membrane damage and LV hypertrophy, both of which may be secondary to inflammatory processes in the myocardium. Evidence for immune activation in a proportion of patients with DCM includes the finding of cardiac endothelial major histocompatibility complex class II expression, disease-specific, cardiac-specific autoantibodies, and increased soluble interleukin 2 receptor levels.[32–35] Immunohistochemical assessment of serial endomyocardial biopsies from patients with DCM has suggested persistent immune activation in almost 40% of patients.[36] Thus, immunohistochemical evidence of persistent immune activation or abnormal CK levels in the circulation may be present in patients who do not have myocardial inflammation by current histologic criteria.[22]

Persistent viral infection could also explain chronic myocardial damage. An association between adverse prognosis and persistent myocardial enterovirus has recently been documented in DCM,[4] although the specificity of this finding was low (only 25%) and no other tissue markers of tissue damage were reported. A greater proportion of patients with adverse clinical outcome had abnormal CK-MB isoforms in the present study. This raises the question of determination of the etiology of myocardial damage in deteriorating patients who do not have demonstrable viral genome, evaluation of tissue markers of immune activation, or elevated CK levels. The measurement of CK-MB isoforms may have an important clinical role in identifying patients who are deteriorating and who might benefit from histologic assessment.

Conclusion

Creatine kinase measurements, in particular CK-MB isoforms, are markers of myocardial damage in a subset of patients with DCM and could be useful in investigating the possibility of persistent myocardial damage in these patients.

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