

Recurrence of Left Ventricular Dysfunction in Patients With Restored Idiopathic Dilated Cardiomyopathy

Jin-Sun Park, MD; Jin-Woo Kim, MD; Kyoung-Woo Seo, MD; Byoung-Joo Choi, MD; So-Yeon Choi, MD, PhD; Myeong-Ho Yoon, MD, PhD; Gyo-Seung Hwang, MD, PhD; Seung-Jea Tahk, MD, PhD; Joon-Han Shin, MD

Department of Cardiology, Ajou University School of Medicine, Suwon, Korea

ABSTRACT

Background: In some patients with nonischemic idiopathic dilated cardiomyopathy (DCM), left ventricular (LV) dysfunction improves spontaneously but can recur. The factors predicting recurrence of LV dysfunction in recovered idiopathic DCM are poorly defined. We investigated the clinical, echocardiographic, and laboratory variables affecting recurrence of LV dysfunction in patients who recovered from DCM.

Hypothesis: The recurrence of LV dysfunction in recovered idiopathic DCM is impacted by clinical, echocardiographic, and laboratory variables.

Methods: The study comprised 85 consecutively enrolled patients (62 males, age 57 ± 16 years) with DCM who achieved a restoration of LV systolic function. Patients were followed up for 50 ± 33 months after recovery from LV dysfunction without discontinuation of standard medication for heart failure with depressed ejection fraction. Clinical, echocardiographic, and laboratory variables were analyzed to identify factors independently associated with recurrence of LV dysfunction.

Results: LV dysfunction recurred in 33 patients (23 males, age 64 ± 12 years). Univariate analysis revealed that age, duration from initial presentation to recovery time, diabetes, and LV end-diastolic dimension (LVEDD) at initial presentation were associated with recurrence of LV dysfunction. Multivariate analysis revealed that only age, diabetes, and LVEDD at initial presentation were independent predictors in patients who recovered from LV dysfunction.

Conclusions: The recurrence of LV dysfunction was significantly correlated with age, presence of diabetes, and LVEDD at initial presentation. Clinicians should consider maintenance of intensive care to patients who recovered from DCM with these factors.

Introduction

In some patients with nonischemic idiopathic dilated cardiomyopathy (DCM), left ventricular (LV) dysfunction improves spontaneously.^{1–3} With standard medical therapies, marked improvement of LV dysfunction is achieved in one-third of patients.³ The clinical course of these patients with recovered idiopathic DCM has not been fully established, although LV dysfunction can recur. The factors predicting recurrence of LV dysfunction in recovered idiopathic DCM are poorly defined. Persistent ultrastructural changes are a suggested etiology of recurrence of LV dysfunction.⁴

In the present study, we investigated the clinical, echocardiographic, and laboratory factors affecting recurrence of LV dysfunction in the patients with recovered idiopathic DCM.

Methods

We consecutively enrolled 85 patients (62 males, age 57 ± 16 years) with idiopathic DCM who achieved restoration of LV systolic function. The medical records of all patients were retrospectively reviewed after informed consent was provided.

At the initial presentation of DCM, LV dysfunction was confirmed by echocardiography according to the recommendations by the American Society of Echocardiography.⁵ LV dysfunction was defined as an ejection fraction (EF) $<40\%$ by echocardiography. Coronary angiography or coronary multidetector computed tomography (MDCT) was performed to exclude LV dysfunction caused by coronary artery disease. We also excluded patients if the LV dysfunction was caused by any of the following: acute myocarditis, stress-induced cardiomyopathy, peripartum cardiomyopathy or cardiomyopathy caused by valvular heart disease, tachycardia, sepsis, metabolic disease, or

The authors have no funding, financial relationships, or conflicts of interest to disclose.

endocrine disorders. All patients were treated with standard medical treatments for heart failure (HF) with depressed EF following current guidelines.⁶

The restoration of LV systolic function was defined as recovery of the EF above 45% and increment of EF \geq 10% compared with the initial EF. As the discontinuation of standard medication for HF with depressed EF might lead to the recurrence of LV dysfunction,⁷ all of the patients continued to be treated with standard medical treatments for HF after recovery from LV dysfunction. Patients were followed up for 50 ± 33 months.

At the presentation of recurrent LV dysfunction, coronary angiography or coronary MDCT was done to exclude LV dysfunction caused by coronary artery disease. All patients were divided into 2 groups according to the recurrence of LV dysfunction: without recurrent LV dysfunction ($n = 52$) and recurrent LV dysfunction ($n = 33$). Clinical, echocardiographic, and laboratory variables were analyzed to identify factors independently associated with recurrence of LV dysfunction. Laboratory variables already identified as predictors of poor outcome in patients with HF were evaluated.^{8–11} The variables included hemoglobin, hematocrit, red cell distribution width, serum sodium, and serum creatinine. The echocardiographic and laboratory findings at the initial presentation of DCM, after recovery of LV dysfunction and at the final follow-up, were monitored.

SPSS version 13.0 software (SPSS Inc., Chicago, IL) was used for all calculations. Data are shown as the mean \pm standard deviation for continuous variables and as percentages for categorical variables. Comparisons were conducted by unpaired Student *t* test. Between the groups, clinical and echocardiographic variables were analyzed to identify factors associated with recurrence of LV dysfunction by univariate analysis. Variables that significantly differed between the 2 groups were entered in multivariate analysis to identify factors independently associated with recurrence of LV dysfunction. Multiple logistic regression analysis was performed to assess independent predictors for recurrence of LV dysfunction. A *P* value <0.05 was considered statistically significant.

Results

Patients were followed up for 50 ± 33 months after recovery from LV dysfunction without discontinuation of standard medication for HF with depressed EF. No patient required readmission related to recurrent HF or optimization of medication from the time of diagnosis of LV dysfunction to the time of recovery. LV dysfunction recurred in 33 patients (23 males, age 64 ± 12 years). Thirty-three patients (39%) were included in the group with recurrent LV dysfunction, and the remaining 52 patients (61%) were included in the group without recurrent LV dysfunction.

Baseline clinical characteristics according to the groups are summarized in Table 1. Patients with recurrent LV dysfunction were older than those without (64 ± 12 vs 53 ± 16 years, $P = 0.001$). Compared to the group without recurrent LV dysfunction, those with recurrent LV dysfunction had a significantly higher rate of diabetes mellitus ($P = 0.005$). Duration from initial presentation to recovery time was significantly longer in the group with

Table 1. Baseline Clinical Characteristics

| Characteristics | Group Without Recurrent LVD (n = 52) | Group With Recurrent LVD (n = 33) | P Value |
|--|--------------------------------------|-----------------------------------|---------|
| Age (years) | 53 \pm 16 | 64 \pm 12 | 0.001 |
| Men | 39 (75%) | 23 (70%) | 0.597 |
| BMI (kg/m ²) | 28.1 \pm 19.4 | 25 \pm 3.8 | 0.376 |
| Hypertension | 25 (48%) | 12 (36%) | 0.117 |
| Diabetes Mellitus | 7 (13%) | 15 (45%) | 0.005 |
| Dyslipidemia | 3 (6%) | 5 (15%) | 0.215 |
| Atrial fibrillation | 9 (17%) | 8 (24%) | 0.623 |
| Bundle branch block | 5 (10%) | 4 (12%) | 0.741 |
| Duration from initial presentation to recovery time (months) | 15.3 \pm 11.5 | 29.9 \pm 28.1 | 0.01 |
| ACE inhibitor or ARB | 48 (92%) | 33 (100%) | 0.623 |
| β -blocker | 15 (29%) | 6 (18%) | 0.1 |
| Aldosterone antagonist | 18 (35%) | 8 (24%) | 0.105 |

ACE, Angiotensin-converting enzyme; ARB, Angiotensin II receptor blocker; BMI, body mass index; LVD, left ventricular dysfunction.

recurrent LV dysfunction than without (29.9 ± 28.1 vs 15.3 ± 11.5 months, $P = 0.01$). There was no significant statistical difference in medical treatments between the 2 groups. No patient underwent intracardiac defibrillator or cardiac resynchronization therapy in either group.

The results of echocardiographic findings are listed in Table 2. At the initial presentation of DCM, the LV end-diastolic dimension (LVEDD) of the group with recurrent LV dysfunction was larger than that of the group without recurrent LV dysfunction (68 ± 8 vs 64 ± 8 mm, $P = 0.014$). LV systolic function measured by EF in patients with recurrent LV dysfunction was similar to patients without recurrent LV dysfunction (27 ± 9 vs $24 \pm 6\%$, $P = 0.063$). There was no statistical difference in the other initial echocardiographic parameters of LV end-systolic dimension, left atrial diameter, and grade of functional mitral regurgitation. After restored LV systolic function, there was no statistical difference in all the echocardiographic parameters between the groups. When the LV dysfunction relapsed in the group with recurrent LV dysfunction, the LV systolic function measured $30 \pm 9\%$ of EF. In the group without recurrent LV dysfunction, the LV systolic function was maintained as $57 \pm 9\%$ of EF at the final echocardiographic follow-up.

The laboratory findings did not show any significant difference at the initial presentation of DCM, after recovery of LV dysfunction and at the final follow-up (Table 3).

Univariate analysis revealed that age, duration from initial presentation to recovery time, presence of diabetes, and LVEDD at initial presentation were associated with recurrence of LV dysfunction. These variables were entered

Table 2. Echocardiographic Characteristics

| Characteristics | Group Without Recurrent LVD (n = 52) | Group With Recurrent LVD (n = 33) | P Value |
|---|--------------------------------------|-----------------------------------|---------|
| Initial presentation of DCM | | | |
| EF, % | 24 ± 6 | 27 ± 9 | 0.063 |
| LVEDD, mm | 64 ± 8 | 68 ± 8 | 0.014 |
| LVESD, mm | 55 ± 8 | 58 ± 10 | 0.185 |
| LA diameter, mm | 48 ± 7 | 47 ± 7 | 0.639 |
| MR, grade | 2 ± 1 | 2 ± 1 | 0.424 |
| After restoration of LV systolic function | | | |
| EF, % | 55 ± 8 | 53 ± 8 | 0.458 |
| LVEDD, mm | 55 ± 7 | 57 ± 6 | 0.195 |
| LVESD, mm | 40 ± 8 | 41 ± 7 | 0.371 |
| LA diameter, mm | 41 ± 7 | 42 ± 8 | 0.722 |
| MR, grade | 1 ± 1 | 1 ± 1 | 0.506 |
| Final echocardiographic follow-up | | | |
| EF, % | 57 ± 9 | 30 ± 9 | <0.001 |
| LVEDD, mm | 53 ± 5 | 64 ± 9 | <0.001 |
| LVESD, mm | 37 ± 6 | 54 ± 10 | <0.001 |
| LA diameter, mm | 43 ± 9 | 47 ± 10 | 0.062 |
| MR, grade | 0 ± 1 | 1 ± 1 | <0.001 |

Abbreviations: DCM, dilated cardiomyopathy; EF, ejection fraction; LA, left atrial; LVD, left ventricular dysfunction; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; MR, mitral regurgitation.

in multivariate analysis to identify factors independently associated with recurrence of LV dysfunction in patients who recovered from LV dysfunction. In a multiple logistic regression model of recurrence of LV dysfunction, age, presence of diabetes, and LVEDD at initial presentation were independent predictors associated with recurrence of LV dysfunction ($P = 0.023$, 0.026 , and 0.006 , respectively; Table 4, Figure 1).

Discussion

The present study demonstrated that age, diabetes, and LVEDD at initial presentation were closely related with recurrence of LV dysfunction in patients who have recovered from idiopathic DCM.

LV systolic function improves or normalizes in one-third of patients with idiopathic DCM.³ Although the prognosis of these patients with recovered idiopathic DCM is excellent,⁸ the prognosis of patients with LV dysfunction that persists or worsens can be dire, despite advances in medical therapies.^{12–14} In some patients recovering from idiopathic DCM, LV dysfunction recurs without any clinically specific reason.⁷ These patients might have a poor prognosis, although their clinical course has not been specifically

Table 3. Laboratory Characteristics

| Characteristics | Group Without Recurrent LVD (n = 52) | Group With Recurrent LVD (n = 33) | P Value |
|---|--------------------------------------|-----------------------------------|---------|
| Initial presentation of DCM | | | |
| Hemoglobin, g/dL | 14 ± 1.8 | 13.2 ± 2.3 | 0.142 |
| Hematocrit, % | 42.1 ± 5.2 | 39.1 ± 6.8 | 0.053 |
| RDW, % | 14.1 ± 1.2 | 13.2 ± 2.3 | 0.101 |
| Sodium, mmol/L | 140.7 ± 3.0 | 140.6 ± 2.4 | 0.932 |
| Creatinine, g/dL | 1.2 ± 0.5 | 1.2 ± 0.8 | 0.830 |
| After restoration of LV systolic function | | | |
| Hemoglobin, g/dL | 14 ± 1.5 | 13.2 ± 2.6 | 0.161 |
| Hematocrit, % | 41.4 ± 4.4 | 38.3 ± 7.5 | 0.092 |
| RDW, % | 12.6 ± 0.3 | 12.9 ± 1.6 | 0.770 |
| Sodium, mmol/L | 139.5 ± 0.6 | 138 ± 3.8 | 0.126 |
| Creatinine, g/dL | 1.2 ± 0.3 | 1.1 ± 0.3 | 0.246 |
| Final echocardiographic follow-up | | | |
| Hemoglobin, g/dL | 14 ± 1.7 | 13.7 ± 1.8 | 0.49 |
| Hematocrit, % | 41.2 ± 5.0 | 40.2 ± 5.6 | 0.473 |
| RDW, % | 13.7 ± 1.0 | 14 ± 1.7 | 0.310 |
| Sodium, mmol/L | 139.5 ± 3.9 | 139.2 ± 3.6 | 0.691 |
| Creatinine, g/dL | 1.4 ± 0.7 | 1.2 ± 0.3 | 0.094 |

Abbreviations: DCM, dilated cardiomyopathy; LVD, left ventricular dysfunction; RDW, red cell distribution width.

Table 4. Multiple Logistic Regression Analysis of the Clinical and Echocardiographic Predictors for Recurrence of Left Ventricular Dysfunction in Patients With Idiopathic Dilated Cardiomyopathy

| Variables | Odds Ratio (95% CI) | P Value |
|---|----------------------|---------|
| Age | 1.060 (1.008–1.114) | 0.023 |
| Duration from initial presentation to recovery time | 1.038 (0.995–1.083) | 0.084 |
| Diabetes mellitus | 4.812 (1.202–19.272) | 0.026 |
| LVEDD at initial echocardiography | 1.178 (1.048–1.325) | 0.006 |

Abbreviations: CI, confidence interval; LVEDD, left ventricular end-diastolic dimension.

investigated. If clinicians could predict these patients in advance, these patients could be managed properly, and clinicians could continue to pay attention to these candidates for the recurrent DCM irrespective of clinical improvement.

In our previous study, LVEDD at initial presentation was significantly correlated with the recovery of LV dysfunction in patients with idiopathic DCM.¹⁵ Increased LV size and volume are powerful predictors of reduced survival.¹⁶ Remodeling of LV is accompanied with histopathologic changes that include loss of myofibrils,^{17,18} myocyte

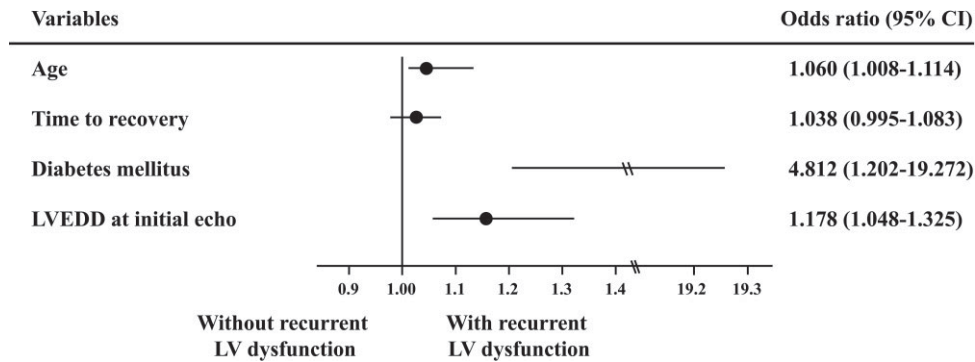


Figure 1. Multivariate analysis of independent predictors for recurrence of left ventricular (LV) dysfunction in patients who recovered from left ventricular dysfunction. Abbreviations: CI, confidence interval; LVEDD, left ventricular end-diastolic dimension.

hypertrophy,¹⁹ and decreased collagen support of adjoining myocytes.²⁰ By increasing LV wall stress, remodeling of LV might aggravate myocardial structural integrity. In the present study, patients with recurrent HF had a larger LVEDD than those without recurrent HF at initial presentation. At initial presentation, larger LV size might intensify the disruption of myocardial ultrastructure. Although it was not confirmed by myocardial biopsy, the initial myocardial ultrastructural disruption might persist after restoration of LV systolic function and result in recurrent LV dysfunction.

Clinical and hemodynamic improvement and normalization of echocardiographic parameters might not present the recovery of idiopathic DCM. In present study, the echocardiographic parameters of both groups showed no statistical difference after restoration of LV systolic function. In 39% of the study population, LV dysfunction recurred. After discontinuation of standard medical treatments for HF with depressed EF, LV dysfunction recurred in patients with restored LV systolic function.⁷ Unexpected sudden death can occur in patients with recovered LV dysfunction.⁴ In animal models, persistent structural deterioration of myocytes were noted after recovery from pacing-induced cardiomyopathy.^{20,21} These data suggest that the myocardial ultrastructural changes persist even after normalization of LV systolic function. The myocardial structural integrity beyond EF representing LV systolic function might be more important in the recurrence of LV dysfunction in patients with recovered idiopathic DCM.

In the present study, aging was a predictor for recurrence of LV dysfunction in recovered idiopathic DCM. Aging myocardium is more susceptible to pathologic damage owing to decline of mitochondrial functional activity.²² Also, the regenerative capacity of myocytes gradually declines with age.²³ Aged myocytes display changes in function and replicative capacity.²⁴ At the ultrastructural level, the complete recovery of DCM might not be achieved, especially in the elderly, even though LV systolic function is normalized. Furthermore, hemodynamic change that occurs with age, such as increase of LV wall tension due to arterial stiffness,²⁵ might influence the recurrence of LV dysfunction in patients with recovered idiopathic DCM.

With change in cardiac energy metabolism, diabetes could lead to impaired cardiac functional reserve capacity.²⁶

Several factors, such as impaired intracellular calcium homeostasis, increased reactive oxygen species generation, and augmented lipid storage leading to lipotoxicity and mitochondrial dysfunction have been suggested as contributors for alteration of cardiac energy metabolism in patients with diabetes.²⁷ Alterations in cardiac energy metabolism might have induced deleterious ultrastructural effects, ultimately leading to the recurrence of LV dysfunction in the patients presently studied.

Although the present study suggests that the main mechanism of recurrence of LV dysfunction in patients with restored idiopathic DCM is ultrastructural disruption beyond EF representing LV systolic function, it was not confirmed by endomyocardial biopsy or cardiac magnetic resonance imaging (MRI). Although a biochemical examination of endomyocardial biopsy could be used as a research tool, it still has limited value.²⁸ Several endomyocardial specimens might not represent the rest of the myocardium.²⁹ Given the limited available evidence, endomyocardial biopsy is recommended for those with specific indications.³⁰ As the population of the present study was not included in appropriate indications according to the guidelines, the endomyocardial biopsy was limited ethically. Cardiac MRI is costly and not widely available. The role of cardiac MRI has not been established in patients with recovered idiopathic DCM. In real practice, clinical and echocardiographic parameters, rather than endomyocardial biopsy or cardiac MRI, could be applied as more useful and easily measurable predictors of the recurrence of LV dysfunction without any ethical and safety concerns. Biomarkers might be useful for predicting recurrence of LV dysfunction. Increasing evidence indicates that biomarkers provide insight into underlying pathophysiologic mechanisms and biologic pathways, while predicting outcomes in patients with idiopathic DCM.³¹ As biomarkers were not measured in most of the present study population, the role of biomarkers for predicting recurrence of LV dysfunction could not be demonstrated. Further studies would be needed to demonstrate the role of biomarkers for predicting recurrence of LV dysfunction.

Selection bias might be a limitation in the present study. The study population was highly selective for those who recovered from idiopathic DCM. As the present study was retrospective, not all patients who recovered from

idiopathic DCM during a certain period were enrolled. Many patients who dropped out during the follow-up could not be evaluated. As clinical and echocardiographic follow-up were usually more frequently done in patients with HF, relatively more patients without recurrent LV dysfunction might have dropped out.

Conclusion

The recurrence of LV dysfunction was significantly correlated with age, presence of diabetes, and LVEDD at initial presentation. Continuous clinical follow-up and maintenance of proper medical treatment should be considered even after restoration of LV dysfunction.

Acknowledgments

The authors thank Seung-Soo Sheen, MD, in Ajou University School of Medicine, Section of Clinical Epidemiology and Biostatistics, Regional Clinical Trial Center, for help with statistical analysis.

References

1. Mann-Rouillard V, Fishbein MC, Naqvi TZ, et al. Frequency of echocardiographic improvement in left ventricular cavity size and contractility in idiopathic-dilated cardiomyopathy. *Am J Cardiol.* 1999;83:131–133, A9–A10.
2. Schwarz F, Mall G, Zebe H, et al. Determinants of survival in patients with congestive cardiomyopathy: quantitative morphologic findings and left ventricular hemodynamics. *Circulation.* 1984;70:923–928.
3. McNamara DM, Holubkov R, Starling RC, et al. Controlled trial of intravenous immune globulin in recent-onset dilated cardiomyopathy. *Circulation.* 2001;103:2254–2259.
4. Nerheim P, Birger-Botkin S, Piracha L, et al. Heart failure and sudden death in patients with tachycardia-induced cardiomyopathy and recurrent tachycardia. *Circulation.* 2004;110:247–252.
5. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr.* 2005;18:1440–1463.
6. McMurray JJ, Adamopoulos S, Anker SD, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *Eur Heart J.* 2012;33:1787–1847.
7. Moon J, Ko YG, Chung N, et al. Recovery and recurrence of left ventricular systolic dysfunction in patients with idiopathic dilated cardiomyopathy. *Can J Cardiol.* 2009;25:e147–e150.
8. Steimle AE, Stevenson LW, Fonarow GC, et al. Prediction of improvement in recent onset cardiomyopathy after referral for heart transplantation. *J Am Coll Cardiol.* 1994;23:553–559.
9. Van Craenenbroeck EM, Pelle AJ, Beckers PJ, et al. Red cell distribution width as a marker of impaired exercise tolerance in patients with chronic heart failure. *Eur J Heart Fail.* 2012;14:54–60.
10. Tang WH, Tong W, Jain A, et al. Evaluation and long-term prognosis of new-onset, transient, and persistent anemia in ambulatory patients with chronic heart failure. *J Am Coll Cardiol.* 2008;51:569–576.

11. Oh C, Chang HJ, Sung JM, et al. Prognostic estimation of advanced heart failure with low left ventricular ejection fraction and wide QRS interval. *Korean Circ J.* 2012;42:659–667.
12. Cetta F, Michels VV. The natural history and spectrum of idiopathic dilated cardiomyopathy, including HIV and peripartum cardiomyopathy. *Curr Opin Cardiol.* 1995;10:332–338.
13. Feild BJ, Baxley WA, Russell RO Jr, et al. Left ventricular function and hypertrophy in cardiomyopathy with depressed ejection fraction. *Circulation.* 1973;47:1022–1031.
14. Ciccoira M, Rossi A, Chiampan A, et al. Identification of high-risk chronic heart failure patients in clinical practice: role of changes in left ventricular function. *Clin Cardiol.* 2012;35:580–584.
15. Shin JH, Choi SY, Yoon MH, et al. Echocardiographic and clinical factors affecting normalization of LV systolic function in patients with cardiomyopathy. *Korean Circ J.* 2001;31:200–209.
16. Pfeffer MA, Pfeffer JM. Ventricular enlargement and reduced survival after myocardial infarction. *Circulation.* 1987;75(5 pt 2):IV93–IV97.
17. Keren A, Gottlieb S, Tzivoni D, et al. Mildly dilated congestive cardiomyopathy. Use of prospective diagnostic criteria and description of the clinical course without heart transplantation. *Circulation.* 1990;81:506–517.
18. Figulla HR, Rahlf G, Nieger M, et al. Spontaneous hemodynamic improvement or stabilization and associated biopsy findings in patients with congestive cardiomyopathy. *Circulation.* 1985;71:1095–1104.
19. Anversa P, Loud AV, Levicky V, et al. Left ventricular failure induced by myocardial infarction. I. Myocyte hypertrophy. *Am J Physiol.* 1985;248(6 pt 2):H876–H882.
20. Spinale FG, Tomita M, Zellner JL, et al. Collagen remodeling and changes in LV function during development and recovery from supraventricular tachycardia. *Am J Physiol.* 1991;261(2 pt 2):H308–H318.
21. Spinale FG, Zellner JL, Tomita M, et al. Relation between ventricular and myocyte remodeling with the development and regression of supraventricular tachycardia-induced cardiomyopathy. *Circ Res.* 1991;69:1058–1067.
22. Azhar G, Gao W, Liu L, et al. Ischemia-reperfusion in the adult mouse heart influence of age. *Exp Gerontol.* 1999;34:699–714.
23. Goldspink DF. Ageing and activity: their effects on the functional reserve capacities of the heart and vascular smooth and skeletal muscles. *Ergonomics.* 2005;48:1334–1351.
24. Sussman MA, Anversa P. Myocardial aging and senescence: where have the stem cells gone? *Annu Rev Physiol.* 2004;66:29–48.
25. Lakatta EG. Changes in cardiovascular function with aging. *Eur Heart J.* 1990;11(suppl C):22–29.
26. Daniels A, van Bilsen M, Janssen BJ, et al. Impaired cardiac functional reserve in type 2 diabetic db/db mice is associated with metabolic, but not structural, remodelling. *Acta Physiol (Oxf).* 2010;200:11–22.
27. An D, Rodrigues B. Role of changes in cardiac metabolism in development of diabetic cardiomyopathy. *Am J Physiol Heart Circ Physiol.* 2006;291:H1489–H1506.
28. MacKay EH, Littler WA, Sleight P. Critical assessment of diagnostic value of endomyocardial biopsy. Assessment of cardiac biopsy. *Br Heart J.* 1978;40:69–78.
29. Baandrup U, Florio RA, Olsen EG. Do endomyocardial biopsies represent the morphology of the rest of the myocardium? A quantitative light microscopic study of single v. multiple biopsies with the King's biopome. *Eur Heart J.* 1982;3:171–178.
30. Cooper LT, Baughman KL, Feldman AM, et al. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology Endorsed by the Heart Failure Society of America and the Heart Failure Association of the European Society of Cardiology. *Eur Heart J.* 2007;28:3076–3093.
31. Gopal DM, Sam F. New and emerging biomarkers in left ventricular systolic dysfunction—insight into dilated cardiomyopathy. *J Cardiovasc Transl Res.* 2013;6:516–527.