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Elevated Intraoperative Expression of Ventricular Myosin Light Chain Predicts Heart Failure after Valve Replacement Surgery

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ABSTRACT

Background: It can be difficult to predict which patients will survive and recover cardiac function after valve replacement surgery. We hypothesized that the expression levels of ventricular myosin light chain (MLCv) might reflect the severity of disease or the extent of irreversible myocardial damage and might be useful for predicting the postoperative course. Thus, the aim of this study was to explore the relationship between MLCv expression in specimens obtained during valve replacement surgery and the postoperative New York Heart Association (NYHA) class.

Methods: The levels of expression of the regulatory MLCv (MLC-2v) and MLC-1v in papillary muscle specimens from 80 patients who underwent valve replacement surgery for rheumatic valvular disease were evaluated by Western blot analysis.

Results: The patients were similar with regard to the intraoperative expression of MLC-1v, regardless of postoperative NYHA class. The preoperative NYHA class, the end-systolic left ventricular internal dimension, and the intraoperative expression of MLC-2v emerged as independent risks factors for a NYHA class status of III/IV at 6 months after surgery, with an area under the receiver operating characteristic curve of 0.862.

Conclusion: The intraoperative level of MLC-2v expression was predictive of the patients' NYHA class after valve replacement surgery. This result suggests that future studies evaluating the use of preoperative specimens (such as biopsy or peripheral blood samples) for measurement of MLC-2v levels could lead to a valuable preoperative tool for the assessment of candidates for valve replacement.

INTRODUCTION

Rheumatic heart disease, the predominant type of valvular heart disease in China, is believed to be caused by anticardiac autoantibodies that are produced subsequent to infection with group A β-hemolytic *Streptococcus* (GABHS) [Cunningham 2000]. It is likely that the M protein on the cell membranes of GABHS cross-reacts with cardiac myosin, which induces T-cell–mediated damage to the heart tissue and valves [Guilherme 2010; Nussinovitch 2011].

The prognosis for patients with severe valvular heart diseases can be very poor without surgical repair [Tarantini 2003]. Surgical replacement of the valve may correct the abnormal hemodynamics caused by valvular heart disease. For example, several studies have shown that surgical replacement of the aortic valve improves long-term survival and the postoperative New York Heart Association (NYHA) class for many, but not all, patients with severe aortic stenosis [Tarantini 2003; Vaquette 2005]. Although the patients had similar clinical manifestations, including the severity and duration of heart failure and the left ventricular dimension, their prognoses after valve replacement were different. In some patients, cardiac dysfunction may persist after valve surgery, and abnormalities of the myocardial contractile protein may still exist [Tarantini 2003]. Whether such abnormalities can lead to further deterioration of cardiac function after surgical treatment is not fully understood, however.

Because of the differences in prognoses, various investigations have focused on identifying factors that predict survival and recovery of cardiac function after surgery. Several preoperative clinical predictive factors have been identified [Tarantini 2003; Vaquette 2005; Flores-Marín 2010]. Furthermore, molecular markers such as natriuretic peptides have been proposed as prognostic indicators of outcome after valve replacement surgery [Della Corte 2008; Bergler-Klein 2009]. Others, however, have reported that natriuretic peptides have prognostic value only among patients who are treated conservatively [Weber 2006]. In general, there remains a need for better prognostic factors to help in the management of patients after valve replacement therapy.

Previous studies have suggested that ventricular myosin light chains (MLCv) play a role in heart failure. Margossian et al [1992] found a decrease in the levels of the regulatory ventricular myosin light chain (MLC-2v) that was associated with diminished ATP binding, steady-state ATP hydrolysis, and affinity for actin in the heart tissues of patients with idiopathic dilated cardiomyopathy. We hypothesized that MLC-2v levels might indicate the severity of disease or the extent of irreversible myocardial damage, prompting us to explore the value of the levels of MLC-2v expression in papillary muscle specimens obtained during surgery as a marker to predict the outcome after surgery in patients with

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rheumatic valvular heart disease. Papillary muscle specimens were used because they are safely and easily obtained during valve replacement [Frustaci 1999].

The aim of this study was to investigate MLC-2v expression in patients with different levels of heart failure due to rheumatic valvular heart disease and to determine its relationship to cardiac function (as defined by the NYHA class) after valve replacement.

METHODS

Patients

From October 2007 to April 2009, 83 patients with rheumatic valvular heart disease (18 men and 65 women) underwent valve replacement operations in our hospital. The mean (±SD) duration of disease was 8.70 ± 9.14 years (range, 1 month to 33 years). The classifications of initial heart function for the patients according to the NYHA were as follows: 5 patients were in NYHA class I, 34 patients were in NYHA class II, 33 patients were NYHA class III, and 11 patients were NYHA class IV. Class definitions are as follows: Class I (mild) features no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitations, or dyspnea (shortness of breath). Class II (mild) is characterized by a slight limitation of physical activity. Such patients are comfortable at rest, but ordinary physical activity leads to fatigue, palpitations, or dyspnea. Class III (moderate) features marked limitation of physical activity. The patient is comfortable at rest but less than ordinary activity causes fatigue, palpitations, or dyspnea. Class IV (severe) patients are unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency present at rest. If any physical activity is undertaken, discomfort is increased. We stratified the patients into 2 groups, mild heart failure (NYHA classes I and II) and moderate to severe heart failure (NYHA classes III and IV), for the analyses performed in this study.

Fifty-nine of the patients underwent mitral valve replacement, and 24 patients underwent combined mitral valve and aortic valve replacement. Three of these patients underwent simultaneous coronary artery bypass surgeries, which led to their exclusion from the study. Additional exclusion criteria included the following: combined coronary artery disease; malignant tumors; hyperthyroidism, hypothyroidism, diabetes, and other endocrine system diseases; chronic liver or renal failure; mental illness; or inability to harvest a cardiac specimen during the operation. Ultimately, 80 patients were enrolled in the study. This study was approved by the Institutional Review Board of the hospital. All patients enrolled in this study signed the informed-consent form.

Assessment of Heart Function

The preoperative left ventricular end-diastolic and end-systolic diameters were measured in the short-axis view of the left ventricle with M-mode color Doppler echocardiography (HP Sonos 2000; Hewlett-Packard, Palo Alto, CA, USA) to obtain the ventricular end-diastolic volume, the end-systolic volume, the ejection fraction, and fractional shortening according to the programmed formula.

Valve Replacement and Extraction of Myocardial MLC

Patients with valvular heart disease underwent valve replacement under cardiopulmonary bypass and cardiac arrest. The mitral valve apparatus was preserved during all replacements, and all surgical treatments were performed by the same surgeon for the purpose of achieving consistent surgical methods and techniques. The papillary muscle attached to the trimmed mitral valve and cord was preserved in liquid nitrogen. The tissue was processed quickly on ice to prevent protein degradation. A specimen of papillary muscle consisting of 50 mg of tissue was combined with 200 mL of RIPA buffer (Beyotime Institute of Biotechnology, Shanghai, P. R. China), homogenized in a mortar at low temperature, subjected to ultrasonic vibration to break the cells, and centrifuged at 21,000*g* at 4°C for 25 minutes. After centrifugation, the supernatant was carefully removed. The protein concentration was determined with the bicinchoninic acid protein assay (Thermo Scientific Company, Waltham, MA, USA), and the concentrations of all protein samples were adjusted to 50 g/20 L. The samples were denatured at 100°C for 5 minutes in 4× loading buffer, divided into small aliquots, and stored at –80°C.

Western Blot Analysis

Fifty micrograms of each protein sample was electrophoresed in a NuPAGE Bis-Tris precast gel (Invitrogen, Carlsbad, CA, USA), the bands were transferred to a polyvinylidene fluoride membrane (Bio-Rad Laboratories, Hercules, CA, USA), and the membrane was washed 3 times with Tris-buffered saline with Tween for 15 minutes. After the membrane was blocked and washed, it was treated with primary antibody (mouse antihuman MLC-1v monoclonal antibody or goat antihuman MLC-2v polyclonal antibody [Santa Cruz Biotechnology, Santa Cruz, CA, USA]; β-actin antibody [Abcam, Cambridge, MA, USA], 1:1000 dilution) at 4C for 16 hours, washed extensively, and treated with horseradish peroxidase–labeled secondary antibodies (1:2000 dilution; Solarbio, Beijing, P. R. China) at room temperature for 2 hours. The peroxidase activity on the membrane was visualized with x-ray film according to a standard chemiluminescence procedure. Quantity One 4.4 software (Bio-Rad Laboratories) was used to determine protein concentrations from the gray bands, and the values were normalized to the β-actin value to control for differences in sample volume. The β-actin content was within the linear interval of the detection method for all samples.

Statistical Analysis

For continuous data, normally distributed variables were expressed as the mean ± SD, and such data were tested with the Student *t* test for 2 independent samples. Variables not normally distributed were expressed as the median and the interquartile range (IQR) and were tested with the Mann-Whitney *U* test. Categorical data were expressed as a count and a percentage and were tested with the Fisher exact test. The McNemar test was used to compare the NYHA class before and after surgery. Univariate and multivariate logistic regression models were used to evaluate risk factors. The variables that reached statistical significance in the corresponding univariate analyses were included in the forward conditional model selection to determine which variables

Table 1. Summary of Patients' Preoperative Demographic and Clinical Characteristics according to the New York Heart Association (NYHA) Class at 6 Months after Surgery*

		NYHA Class at 6 Months after Surgery		
Characteristic	Total (n = 80)	I/II (n = 64)	III/IV (n = 16)	*P*†
Age, y‡	51.7 ± 9.6	51.7 ± 10.0	51.7 ± 8.2	1.000
Sex, n§				.292
Male	16 (20.0%)	11 (17.2%)	5 (31.3%)	
Female	64 (80.0%)	53 (82.8%)	11 (68.8%)	
Preoperative NYHA class, n§				<.001
I/II	37 (46.3%)	36 (56.3%)	1 (6.3%)	
III/IV	43 (53.8%)	28 (43.8%)	15 (93.8%)	
Surgery type§				.128
DVR	24 (30.0%)	22 (34.4%)	2 (12.5%)	
MVR	56 (70.0%)	42 (65.6%)	14 (87.5%)	
Disease duration, y‖	6.0 (2.0-10.0)	5.0 (1.0-10.0)	10.0 (6.5-19.5)	.006
EF, %‡	60.7 ± 8.9	61.5 ± 8.6	57.4 ± 9.4	.102
FS, %‡	33.1 ± 6.4	33.7 ± 6.3	30.8 ± 6.6	.107
LVID, cm‡				
End-diastolic	5.2 ± 0.8	5.1 ± 0.8	5.4 ± 1.1	.186
End-systolic	3.5 ± 0.6	3.4 ± 0.6	3.8 ± 0.8	.037
IVST, cm‖				
End-diastolic	0.9 (0.8-1.0)	0.9 (0.8-1.0)	0.9 (0.8-1.0)	.791
End-systolic	1.3 (1.1-1.4)	1.3 (1.1-1.4)	1.2 (1.1-1.4)	.201
LVWT, cm‡				
End-diastolic	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	.631
End-systolic	1.3 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	.136
SV, mL‖	72.1 (58.8-92.9)	72.1 (60.1-92.9)	72.0 (57.0-88.2)	.846
PAP, mm Hg‡	57.1 ± 14.7	53.0 ± 11.3	67.4 ± 17.4	.024
MLC-1v‖¶	0.5 (0.2-1.1)	0.5 (0.2-1.0)	0.7 (0.2-1.1)	.986
MLC-2v‖¶	1.0 (0.4-2.4)	0.8 (0.3-2.1)	1.8 (1.3-5.5)	.003
MLC-1v/MLC-2v ratio‖	0.5 (0.2-1.1)	0.7 (0.2-1.4)	0.2 (0.2-0.4)	.001

*DVR indicates combined aortic and mitral ("double") valve replacement; MVR, mitral valve replacement; EF, ejection fraction; FS, fractional shortening; LVID, left ventricular internal dimension; IVST, interventricular septal thickness; LVWT, left ventricular wall thickness; SV, stroke volume; PAP, pulmonary arterial pressure; MLC-1v, ventricular myosin light chain 1; MLC-2v, ventricular myosin light chain 2.

†A *P* value <.05 indicates a statistically significant difference between patients in NYHA class II and patients in NYHA class >II at 6 months after surgery.

‡Data are normally distributed and expressed as the mean ± SD.

§Categorical data are expressed as a count (percentage).

‖Data are not normally distributed and are expressed as the median (interquartile range).

¶MLC-1v and MLC-2v data are expressed in relative units (normalized to β-actin expression).

should be included into the multivariate model. The results of logistic regression models were summarized by a receiver operating characteristic (ROC) curve, as well as the odds ratio (OR) with the corresponding 95% confidence interval (CI). All statistical analyses were 2-tailed and performed with SPSS statistical analysis software (version 15.0; SPSS, Chicago, IL, USA). Statistical significance was set at a *P* level of .05.

RESULTS

Patients' Heart Functions according to NYHA Class

Eighty patients with rheumatic valvular heart disease who met the inclusion and exclusion criteria were enrolled in the study. The patients comprised 16 men (20.0%) and 64 women (80.0%), with a mean age of 51.7 ± 9.6 years. Fifty-six patients (70%) underwent mitral valve replacement, and

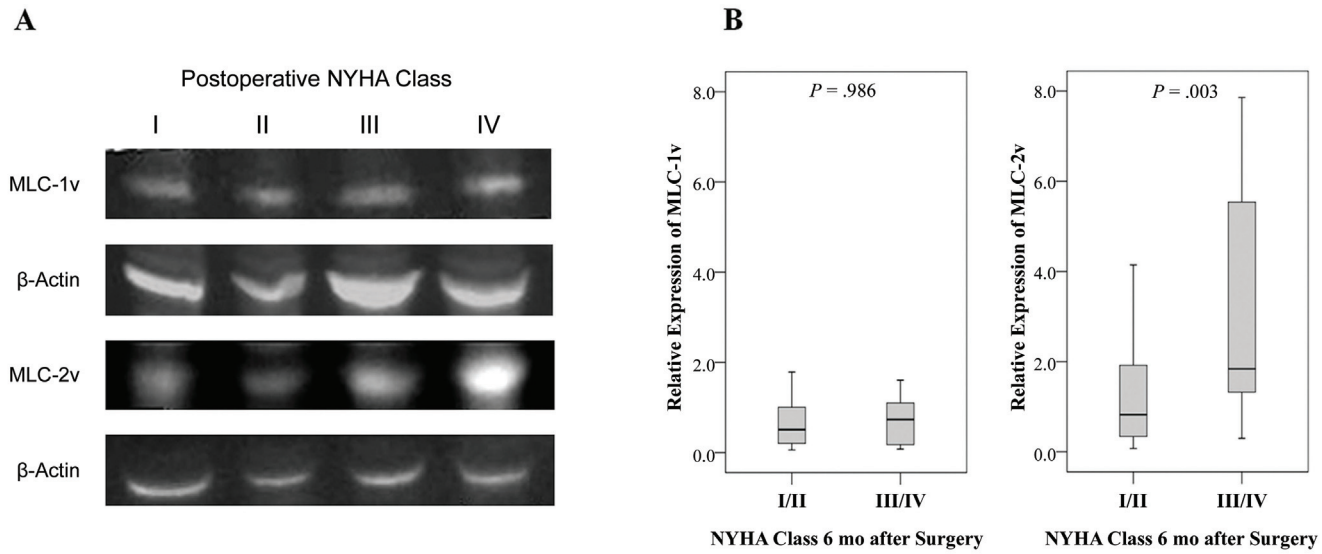

Figure 1. Levels of expression of ventricular myosin light chains (MLC-1v and MLC-2v) according to New York Heart Association (NYHA) class at 6 months after surgery. Protein was extracted from papillary muscle attached to the trimmed mitral valve and cord of patients who underwent valve replacement surgery. A, Levels of MLC-1v and MLC-2v expression were evaluated by Western blot analysis. B, Quantification of MLV-1v and MLC-2v expression was obtained by normalizing relative to β-actin expression. Data are expressed as the median and interquartile range.

24 (30%) underwent combined aortic and mitral ("double") valve replacement. At 6 months after valve replacement surgery, 28 of the 43 patients who were in NYHA class III/IV preoperatively had improved to class I/II postoperatively, whereas the other 15 patients remained in class III/IV. Only 1 patient experienced a decline in NYHA class (from class I/II to class III/IV; $P < .001$). Thus, 20.0% of the patients were in class III/IV (moderate to severe heart failure) postoperatively, compared with 53.8% preoperatively (Table 1).

Clinical Characteristics and MLC-1v and MLC-2v Expression in Patients with Mild versus Moderate to Severe Heart Failure after Surgery

Compared with the patients who had mild heart failure after surgery, the patients who had heart failure classified as moderate to severe after surgery had significantly longer disease durations (median, 10.0 years [IQR, 6.5-19.5 years] versus 5.0 years [IQR, 1.0-10.0 years]; $P = .006$), a greater preoperative end-systolic left ventricular internal dimension (LVID) (3.8 ± 0.8 cm versus 3.4 ± 0.6 cm, $P = .037$), a higher preoperative pulmonary arterial pressure (67.4 ± 17.4 mm Hg versus 53.0 ± 11.3 mm Hg, $P = .024$). No significant differences were noted between these 2 groups with respect to preoperative interventricular septal thickness, left ventricular wall thickness, stroke volume, ejection fraction, and fractional shortening.

Papillary muscles were collected intraoperatively for protein analysis of MLC-1v and MLC-2v (Figure 1A). The patients in class III/IV and those in class I/II after surgery were not significantly different with respect to the expression of MLC-1v (Table 1, Figure 1). Quantification experiments indicated higher MLC-2v expression (median, 1.8 units [IQR, 1.3-5.5 units] versus 0.8 units [IQR, 0.3-2.1 units]; $P = .003$) and lower MLC-1v/MLC-2v ratios (median, 0.2 [IQR, 0.2-0.4] versus 0.7 [IQR, 0.2-1.4]; $P = .001$) in patients in NYHA class III/IV after surgery, compared with patients in class I/II (Table 1 and Figure 1B).

Patients' Pathologies according to NYHA Class

There was no significant difference between the 2 groups in the patients' pathologies except for aortic stenosis. No patient in NYHA class III/IV had aortic stenosis, but 34.4% of the patients in the NYHA class I/II had mild to severe aortic stenosis ($P = .008$; Table 2).

Risk Factors for Moderate to Severe Heart Failure after Surgery

The univariate analysis indicated that various factors, including preoperative NYHA class, disease duration, end-systolic LVID, pulmonary arterial pressure, intraoperative MLC-2v expression, and the MLC-1v/MLC-2v ratio, had significant effects on the risk of moderate to severe heart failure after surgery; however, only the preoperative NYHA class, the end-systolic LVID, and the intraoperative expression of MLC-2v emerged as independent risk factors in the multivariate logistic regression model (Table 3). In the multivariate logistic regression model in which the conditions of the other 2 risk factors were fixed, the patients who were classified as having moderate to severe heart failure before surgery had a significantly higher risk for moderate to severe heart failure after surgery (OR, 20.21; 95% CI, 2.29-178.11; $P = .007$). The risk for moderate to severe heart failure after surgery was increased (OR, 4.16; 95% CI, 1.31-13.16; $P = .015$) along with an increase in the end-systolic LVID.

Table 2. Summary of the Patients' Pathologies according to New York Heart Association (NYHA) Class at 6 Months after Surgery

		NYHA Class at 6 Months after Surgery		
Pathology	Total	I-II (n = 64)	III-IV (n = 16)	*P**
Mitral stenosis, n				.241†
Severe	20 (25.0%)	15 (23.4%)	5 (31.3%)	
Moderate-to-severe	10 (12.5%)	10 (15.6%)	0 (0.0%)	
Moderate	30 (37.5%)	27 (42.2%)	3 (18.8%)	
Mild-to-moderate	1 (1.3%)	0 (0.0%)	1 (6.3%)	
Mild	7 (8.8%)	4 (6.3%)	3 (18.8%)	
None	12 (15.0%)	8 (12.5%)	4 (25.0%)	
Mitral insufficiency, n				.108†
Severe	3 (3.8%)	1 (1.6%)	2 (12.5%)	
Moderate-to-severe	8 (10.0%)	6 (9.4%)	2 (12.5%)	
Moderate	11 (13.8%)	7 (10.9%)	4 (25.0%)	
Mild-to-moderate	3 (3.8%)	3 (4.7%)	0 (0.0%)	
Mild	37 (46.3%)	32 (50.0%)	5 (31.3%)	
None	18 (22.5%)	15 (23.4%)	3 (18.8%)	
Aortic stenosis, n				.008†
Severe	1 (1.3%)	1 (1.6%)	0 (0.0%)	
Moderate-to-severe	1 (1.3%)	1 (1.6%)	0 (0.0%)	
Moderate	7 (8.8%)	7 (10.9%)	0 (0.0%)	
Mild-to-moderate	13 (16.3%)	13 (20.3%)	0 (0.0%)	
None	58 (72.5%)	42 (65.6%)	16 (100.0%)	
Aortic insufficiency, n				312†
Severe	1 (1.3%)	0 (0.0%)	1 (6.3%)	
Moderate-to-severe	18 (22.5%)	16 (25.0%)	2 (12.5%)	
Moderate	3 (3.8%)	3 (4.7%)	0 (0.0%)	
Mild-to-moderate	9 (11.3%)	8 (12.5%)	1 (6.3%)	
None	49 (61.3%)	37 (57.8%)	12 (75.0%)	
Mitral valve prolapse, n	8 (10.0%)	6 (9.4%)	2 (12.5%)	.657‡
Mitral regurgitation, n	1 (1.3%)	1 (1.6%)	0 (0.0%)	1.000‡
Mitral aortic calcification, n	3 (3.8%)	2 (3.1%)	1 (6.3%)	.493‡

*A *P* value <.05 indicates a statistically significant difference between patients in NYHA class II and patients in NYHA class >II at 6 months after surgery.
†Mann-Whitney *U* test.
‡Fisher exact test.

In addition, a higher intraoperative expression of MLC-2v was associated with an increased risk for moderate to severe heart failure after surgery (OR, 1.34; 95% CI, 1.03-1.75). The accuracy of the predictions of the multivariate logistic regression model was evaluated by determining the area under the ROC curve, which reached 0.862 (95% CI, 0.772-0.953) (Figure 2).

DISCUSSION

In this study, we examined the relationship between the level of MLC-2v expression at the time of surgery and cardiac function (as indicated by the NYHA class) after valve replacement surgery. We identified the preoperative NYHA class, the end-systolic LVID, and the intraoperative level of MLC-2v

Table 3. Risk Factors for New York Heart Association (NYHA) Class III/IV Heart Failure after Surgery (Univariate and Multivariate Logistic Regression Models)*

	Univariate Analysis		Multivariate Analysis	
Factor	OR (95% CI)	*P*†	OR (95% CI)	*P*†
Age	1.00 (0.94-1.06)	1.000		
Male sex	2.19 (0.63-7.57)	.216		
Preoperative NYHA class (III/IV versus I/II)	19.29 (2.40-154.93)	.005	20.21 (2.29-178.11)	.007
Surgery type (DVR versus MVR)	3.67 (0.76-17.60)	.105		
Disease duration in years	1.08 (1.01-1.14)	.014		
EF	0.95 (0.89-1.01)	.106		
FS	0.93 (0.85-1.02)	.111		
LVID (cm)				
End-diastolic	1.53 (0.81-2.91)	.189		
End-systolic	2.39 (1.02-5.56)	.044	4.16 (1.31-13.16)	.015
IVST (cm)				
End-diastolic	0.87 (0.04-20.05)	.931		
End-systolic	0.14 (0.01-2.49)	.179		
LVWT (cm)				
End-diastolic	8.71 (0.50-152.96)	.139		
End-systolic	0.57 (0.02-14.02)	.732		
SV (mL)	1.00 (0.99-1.02)	.734		
PAP (mm Hg)	1.08 (1.02-1.15)	.012		
MLC-1v‡	0.79 (0.39-1.62)	.520		
MLC-2v‡	1.31 (1.06-1.63)	.013	1.34 (1.03-1.75)	.029
MLC-1v/MLC-2v ratio	0.05 (0.01-0.49)	.010		
Pathology				
Mitral stenosis (yes versus no)	0.43 (0.11-1.66)	.220		
Mitral insufficiency (yes versus no)	1.33 (0.33-5.28)	.689		
Aortic stenosis (yes versus no)	NA	.998		
Aortic insufficiency (yes versus no)	0.46 (0.13-1.57)	.214		
Mitral valve prolapse (yes versus no)	1.38 (0.25-7.59)	.710		
Mitral regurgitation (yes versus no)	NA	1.000		
Mitral aortic calcification (yes versus no)	2.07 (0.18-24.33)	.564		

*OR indicates odds ratio; CI, confidence interval; NA, not applicable because of the absence of patients in class III/IV. Other abbreviations are expanded in the first footnote to Table 1.

†A *P* value <.05 indicates a statistically significant effect on the risk of NYHA class III/IV.

‡MLC-1v and MLC-2v data were expressed in relative units (normalized to β-actin expression).

expression as significant independent risk factors for moderate to severe heart failure after surgery. The accuracy of the predictions of the multivariate logistic regression model was good, as indicated by the area under the ROC curve.

Cardiac dysfunction may persist after valve surgery in some patients. For example, Tarantini et al reported that only 85% of the patients in their study achieved a significant improvement in NYHA functional class [Tarantini 2003]. Preoperative clinical factors that predict survival and recovery of the left ventricular systolic function after surgery include a high mean preoperative transaortic gradient, a smaller degree of cardiomegaly, and a lower left ventricular end-systolic volume index [Tarantini 2003; Vaquette 2005], whereas variables that predict a lack of improvement include a previous

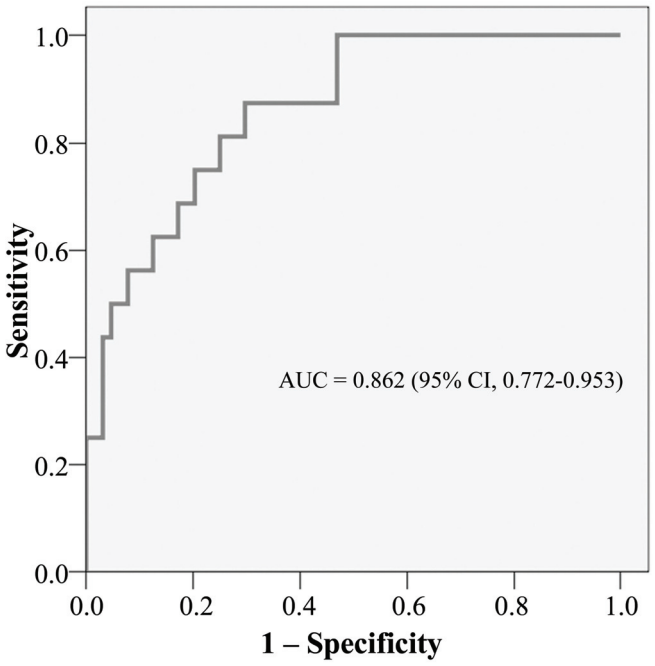

Figure 2. Receiver operating characteristic (ROC) curve for the predictive performance of the regulatory ventricular myosin light chain (MLC-2v). The results of the multivariate analysis shown in Table 2 were summarized by an ROC curve and the odds ratios with the corresponding 95% confidence interval (CI), with corrections for preoperative New York Heart Association class and end-systolic left ventricular internal dimension. AUC indicates area under the ROC curve.

acute myocardial infarction and coronary lesions without revascularization [Flores-Marín 2010]. These findings suggest that patients who do poorly after valve replacement may be those with more prevalent myocardial damage.

In addition to clinical variables, various molecular markers have been proposed as prognostic indicators of outcome after valve replacement surgery [Goto 2003; Sato 2004]. Natriuretic peptides have prognostic value for patients with aortic stenosis and severe aortic regurgitation [Della Corte 2008; Bergler-Klein 2009], but only among patients who are treated conservatively [Weber 2006]. Whether a correlation or covariation exists between MLC-2v levels and those of previously established markers, such as natriuretic peptides, is an interesting topic for future investigation.

Given the important role of myosin in heart contraction, it is not surprising that alterations in the expression and phosphorylation of myosin components have been demonstrated in various diseases of the heart. Studies of patients with congenital heart disease have shown that the MLC-1 isoforms are important for regulating the contractility of the heart [Morano 1996].

MLC-2v contributes to the formation of sarcomeres and increasing the sensitivity of the muscle to Ca^{2+} in the myocardium [Chen 1998; Olsson 2004]. In the zebrafish model, MLC-2v is required for stabilization of thick myosin filaments and contractility [Rottbauer 2006]. The removal of MLC-2v from skinned skeletal muscle fibers produces a decreased high-velocity phase of unloaded shortening, possibly because the dissociation of the myosin cross-bridge from actin is slowed [Hoffmann 1990]. These results are consistent with the conclusion that a reduction in MLC-2v contributes to the contractile failure observed in patients with heart failure [Solaro 1992] and suggest that intraoperative MLC-2v levels might indicate the severity of disease or the extent of irreversible myocardial damage. In this study, therefore, we explored the value of MLC-2v expression levels as a marker for predicting outcome after surgery.

The novel finding of our study was that the MLC-2v level at the time of surgery was a significant independent predictor of moderate to severe heart failure 6 months after valve replacement in patients with rheumatic valvular disease. This result is in agreement with the many previously reported lines of evidence suggesting that MLC-2v has an important role in the pathophysiology of heart failure. A report on gene expression patterns in patients with aortic valve stenosis showed that MLC-2v expression increased proportionally with the transvalvular gradient, especially in women [Villar 2009]. Greenberg et al [2010] have demonstrated that mutations in MLC-2 are linked to cardiomyopathy.

The regulatory function of MLC-2v is mediated by phosphorylation catalyzed by a specific cardiac kinase, myosin light chain kinase 3 (MLCK3), the expression of which is up-regulated in myocardia from patients with heart failure [Seguchi 2007]. The up-regulation of MLCK3 has been suggested to compensate for the lower expression and reduced phosphorylation of MLC-2v found in patients with heart failure. Consistent with this hypothesis, Jacques et al [2008] have shown that the level of MLC-2 phosphorylation in hearts from patients with hypertrophic obstructive cardiomyopathy was 51% of the level in donor hearts. A gradient of MLC-2v phosphorylation in the cardiac wall has been reported to be responsible for the pattern of cardiac contraction and generation of torsion [Davis 2001]. Furthermore, MLCK-induced phosphorylation of MLC-2 has been shown to increase the sensitivity of myofilaments to Ca^{2+} in the atrium and ventricle [Kockskämper 2008]; however, much remains to be learned about the in vivo ventricular function of MLC-2v phosphorylation [Davis 2002; Dias 2006]. In future studies, it will be interesting to evaluate the ratio of phosphorylated MLC-2v to total MLC-2v and the level of MLCK3 expression.

Sütsch et al [1992] found that the expression of the atrial essential light chain (MLC-1a) was elevated in the left ventricle of patients with aortic valve disease, compared with normal ventricular tissue (which does not express the atrial isoform in adults), and that this expression was correlated with the hemodynamic parameters of the hearts before and after valve replacement [Sütsch 1992]. Furthermore, genotype analyses of patients with familial hypertrophic cardiomyopathy have confirmed the important functional roles of both the atrial and ventricular forms of MLC-1 [Lee 2001; Hernandez 2007]. Our observation that the patients in our study did not differ with respect to MLC-1v expression is consistent with the findings of Sütsch et al, who showed that under the conditions of pathologic hemodynamics in their

patients with aortic stenosis or insufficiency, the total content of MLC-1 in the ventricle increased because of the higher MLC-1a level. The MLC-1v content did not change, however [Sütsch 1992].

A limitation of this study is the lack of homogeneity in disease stages among our patients who underwent valve replacement. This feature may have contributed to the relatively large variation in the levels of MLC-2v expression in this study. It may also account for the difference between our results and those of Trahair et al, who found that patients with end-stage heart failure due to dilated cardiomyopathy had a decrease in the expression of MLC-1v protein but not in MLC-2v [Trahair 1993]. The only significant difference in pathology between the patients in the 2 groups in our study, however, was the higher prevalence of aortic stenosis in the patients with mild heart failure, although we did notice that some patients in NYHA class III/IV had aortic stenosis. The reason for this discrepancy remains to be answered. Another limitation is that we evaluated the expression of only 2 myosin components (MLC-1v and MLC-2v) in this study because of their clear relationship with cardiac contractility. In the future, we plan to use proteomic tools to detect the differential expression of multiple genes in valvular diseases, which could enhance the strength of prediction. Finally, our requirement for tissue specimens to determine the expression levels of MLC-2v limited the number of patients in the study to those who were undergoing valve replacement surgery. This limitation had 2 consequences: The sample size was relatively small, and the results of our cohort could not be compared with those of healthy individuals or patients who did not undergo surgery. Future studies evaluating the use of preoperative specimens, such as biopsy or blood samples, for the measurement of MLC-2v levels could lead to a valuable preoperative tool for assessing candidates for valve replacement. A study involving a much large population is necessary to confirm our results.

CONCLUSION

Our study identified preoperative NYHA class, end-systolic LVID, and the intraoperative level of MLC-2v expression as significant independent risk factors for moderate to severe heart failure after valve replacement surgery. This report is the first to analyze the prognostic value of MLC-2v. A determination of the level of MLC-2v expression requires access to tissue specimens, which potentially limits the application to the management of patients after valve replacement surgery. Additional studies with a larger number of patients are needed to validate our findings and to determine the effects of etiology and severity of disease on the expression of MLC-2v.

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