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PRECLINICAL RESEARCH

Quantification of DNA Damage in Heart Tissue as a Novel Prediction Tool for Therapeutic Prognosis of Patients With Dilated Cardiomyopathy

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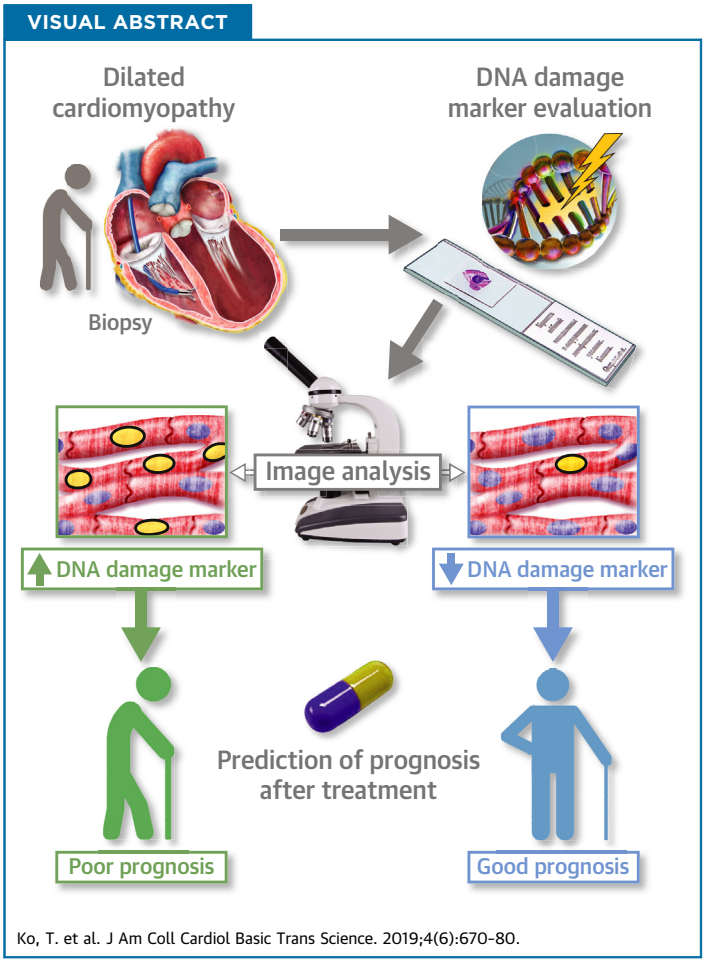
VISUAL ABSTRACT



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HIGHLIGHTS

- **Patients with dilated cardiomyopathy who achieved LVRR have a favorable prognosis, but it is still difficult to precisely predict LVRR in the clinical setting.**
- **Immunostaining of DNA damage markers such as PAR in biopsy specimens from patients with dilated cardiomyopathy revealed that patients with LVRR showed a significantly smaller proportion of PAR-positive nuclei compared with those without LVRR.**
- **The high proportion of PAR-positive nuclei was an independent prognostic factor for LVRR. Besides, it can predict clinical prognosis (death, heart transplantation, and ventricular assist device implantation) with good sensitivity and specificity.**

ABBREVIATIONS AND ACRONYMS

BMI = body mass index

BNP = B-type natriuretic peptide

CI = confidence interval

DAPI = 4′,6-diamidino-2-phenylindole

DCM = dilated cardiomyopathy

IQR = interquartile range

LVAD = left ventricular assist device

LVEF = left ventricular ejection fraction

LVRR = left ventricular reverse remodeling

NYHA = New York Heart Association

ROC = receiver-operating characteristic

PAR = poly(ADP-ribose)

WGA = wheat germ agglutinin

SUMMARY

This study evaluated myocardial nuclear staining for the DNA damage markers poly(ADP-ribose) (PAR) and γ-H2A.X in 58 patients with dilated cardiomyopathy. Patients with left ventricular reverse remodeling (LVRR) showed a significantly smaller proportion of PAR-positive nuclei and γ-H2A.X-positive nuclei in biopsy specimens compared with those without LVRR. Propensity analysis showed that the proportion of both PAR-positive and γ-H2A.X-positive nuclei were independent prognostic factors for LVRR. In conclusion, we showed the utility of DNA damage-marker staining to predict the probability of LVRR, thus revealing a novel prognostic predictor of medical therapy for dilated cardiomyopathy.

Heart failure is a global problem, with an estimated prevalence of 38 million patients worldwide, and a major cause of morbidity and mortality despite advances in cardiovascular therapy throughout the past decades (1,2). Among the various etiologies of heart failure, dilated cardiomyopathy (DCM) is a common cause. DCM is typically diagnosed by left ventricular dilation and impaired systolic function without any known cause (e.g., pressure overload or coronary artery disease) sufficient to explain the myocardial dysfunction (3). Common pharmacological and device therapies such as β-blockers, renin-angiotensin-aldosterone system inhibitors, and cardiac resynchronization therapy induce left ventricular reverse remodeling (LVRR), characterized by a decrease in left ventricular volume and improvement in systolic function in a certain population of patients with DCM (4). In many clinical trials studying patients with DCM, mortality rates decreased with increasing left ventricular ejection fraction (LVEF) and decreasing left ventricular end-diastolic and end-systolic volumes (4–6).

The potential for left ventricular recovery is probably intrinsically conserved in the setting of cardiac

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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dysfunction, and even in the presence of apparently severe myocardial dysfunction (7). However, the recovery of left ventricular function is not universal, occurring in only about 40% of patients with DCM (4,5). Therefore, a major unmet need in the clinical setting is the identification of patients who retain this potential for heart function recovery. Even in the United States, a shortage of donor hearts is a critical problem. Over the past 2 decades, there have been increasingly long waiting times for heart transplantation because the number of available hearts has decreased substantially, from 38% in 2000 to 32% in 2010 (8). In Japan, DCM is the primary cause of end-stage heart failure requiring heart transplantation. The waiting time for patients is much longer in Japan than in other countries, and a substantial proportion of patients die while on the waiting list. If we can anticipate the patients unlikely to achieve remodeling, then these patients can be preferentially referred for mechanical circulatory support and heart transplantation at an earlier stage. The long-term management and prognostic stratification of patients with DCM would benefit from the identification of reliable clinical predictors of LVRR to optimize the treatment strategy and improve patient prognosis.

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Many studies have attempted to identify a predictor of LVRR in patients with DCM (9–11). At present, hemodynamic parameters such as blood pressure, echocardiographic parameters such as left ventricular end-diastolic diameter, and interstitial fibrosis evaluated by late gadolinium enhancement of cardiac magnetic resonance are reported as useful predictors of LVRR. However, common examinations such as blood pressure measurement and echocardiography are not strong predictors of LVRR. Likewise, late gadolinium enhancement evaluation by cardiac magnetic resonance is burdened with the issue of false positives and cannot be performed in patients with renal dysfunction. Therefore, we still lack an accurate method to predict LVRR in patients with DCM (7).

In previous studies of animal models, accumulation of unrepaired oxidative DNA damage was observed in the failing heart (12,13). Our group has previously reported that the accumulation of unrepaired DNA single-strand breaks plays a causative role in the pathogenesis of heart failure (14). Furthermore, using single-cardiomyocyte RNA sequencing, we have also reported that p53 in cardiomyocytes increases cell-to-cell transcriptional heterogeneity and drives pathogenic gene programs, which shows how the accumulation of DNA damage leads to heart failure (15). However, all of these studies were conducted using animal models, and there have been few studies on DNA damage in human heart failure.

Poly(ADP-ribose) (PAR) is a major marker of DNA damage; it solely regulates diverse biological processes known as DNA damage responses (16). The DNA damage responses include DNA repair, chromatin remodeling, transcription, and cell death (17). A recent report by Hoch et al. (18) has shown that PAR in brain tissues could reflect the disease severity of cerebellar ataxia caused by a mutation of *XRCC1*, a gene involved in DNA single-strand break repair. This report raises the possibility that PAR staining could be used to measure DNA damage in human tissues. In the present study, we conducted immunofluorescence staining of PAR in endomyocardial biopsy specimens obtained from patients with DCM to confirm whether there is a DNA damage signature in the failing human heart. We also aimed to verify the utility of immunostaining of DNA damage markers for the prediction of response to heart failure therapy in patients with DCM.

METHODS

STUDY POPULATION AND DESIGN. We retrospectively enrolled patients who were diagnosed with DCM and underwent endomyocardial biopsy between 2009 and 2016 at the University of Tokyo Hospital, a tertiary referral center in Japan for cardiomyopathies. No statistical methods were used to estimate a priori sample size. The DCM diagnosis was made according to current guidelines and based on various modalities including coronary angiography, echocardiography, and endomyocardial biopsy (19,20). As this study is intended to evaluate the utility of immunostaining DNA damage markers in biopsy specimens for the prognostic stratification of patients, patients who have already received optimal medical therapy at the time of biopsy were excluded. According to the general consensus, β-blockers are one of the most important and established medical agents used as the standard therapeutic strategy to achieve LVRR in DCM. As β-blockers are known to improve heart function in a dose-dependent manner, we excluded patients who had already received therapeutic doses of β-blockers (equivalents of carvedilol >5 mg) at the time of biopsy.

In advance, we excluded hemodynamically-unstable patients who had received intravenous catecholamine infusion therapy or mechanical support therapy such as intra-aortic balloon pump therapy and percutaneous cardiopulmonary support within the 30 days preceding biopsy, and patients treated

with left ventricular assist device (LVAD) implantation or heart transplantation. As this study is intended to evaluate the utility of immunostaining DNA damage markers in biopsy specimens for the prognostic stratification of patients, patients who have already received optimal medical therapy at the time of biopsy were excluded. According to the general consensus, β-blockers are 1 of the most important and established medical agents used as the standard therapeutic strategy to achieve LVRR in DCM. As β-blockers are known to improve heart function in a dose-dependent manner, we excluded patients who had already received therapeutic doses of β-blockers (equivalents of carvedilol >5 mg) at the time of biopsy.

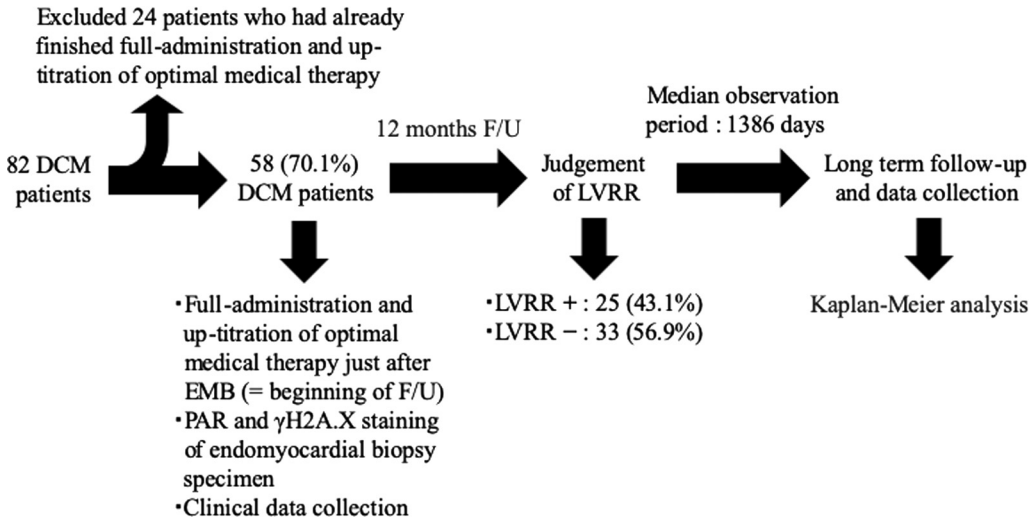
The collection of clinical data, the conduction of immunofluorescence staining for PAR, and the combining of those data to perform statistical analyses were all independently performed by 3 different researchers. The endpoint was a combined endpoint, defined as a composite outcome of death, ventricular assist device implantation, and heart transplantation. Optimal medical therapy for heart failure, including the administration of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, antimineralocorticoids, and up-titration of β-blocker dosages, were initiated shortly after the patients underwent endomyocardial biopsy and a diagnosis of DCM was confirmed. LVRR was defined as an absolute increase in LVEF from ≥10% to a final value of >35%, accompanied by a decrease in left ventricular end-diastolic diameter ≥10% assessed by echocardiography 12 months after the initiation of optimal medical therapy (10). Patients who had events defined as combined endpoint before 12 months after initiation of therapy were categorized into the LVRR-negative group.

Our study was conducted according to the guidelines of the Declaration of Helsinki. Previous approval was obtained from the institutional research ethics committee, which waived the need for individual informed consent (Approval Number 11801).

CLINICAL MEASUREMENTS. Demographic data previously reported to be associated with LVRR in patients with DCM were collected during chart reviews. Demographic variables included age, sex, body mass index (BMI), hemodynamic status at presentation (blood pressure, heart rate), family history, past medical history, medication, and device therapy information. Familial DCM was defined as patients with at least 1 additional family member with DCM. We also reviewed laboratory data and clinical parameters such as electrocardiography and echocardiography collected both at the time of biopsy and 12 months after the initiation of optimal medical therapy.

IMMUNOHISTOCHEMICAL STUDIES. Immunofluorescence staining was used to investigate the expression of PAR and γ-H2A.X in formalin-fixed, paraffin-embedded biopsy specimens from patients with DCM. Briefly, 4-μm sections were cut from paraffin blocks and placed onto slides. After deparaffinization and rehydration, antigens were unmasked by boiling the slides for 20 min in Dako S1699 antigen retrieval solution (Agilent, Santa Clara, California) using an MI-33 microwave processor (Azumaya Co. Ltd., Tokyo, Japan). Slides were blocked in 5% normal goat serum for 60 min at room temperature and subsequently incubated with anti-PAR polymer antibody (ab14459, 1:100, Abcam, Cambridge, Massachusetts) overnight. After washing with phosphate-buffered saline, samples were stained with anti-mouse IgG-Alexa 647 (1:300, Thermo Fisher Scientific, Waltham, Massachusetts) for 1 h at room temperature. Cell membranes and nuclei were counterstained with wheat germ agglutinin (WGA)-Alexa 488 (1:200; Thermo Fisher Scientific), and 4′,6-diamidino-2-phenylindole (DAPI) (1:1,000; Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Other antibodies and dyes used in this study include γ-H2A.X (ab81299, 1:200, Abcam), γ-H2A.X (MA1-2022, 1:200, Thermo Fisher Scientific), WGA-Alexa 350 (1:200; Thermo Fisher Scientific), vimentin (ab92547, 1:200, Abcam), PECAM1 (HPA004690, 1:100, Sigma-Aldrich), and anti-mouse IgG-Alexa 488 and 594 (1:300, Thermo Fisher Scientific). We used 2 sections from the residual biopsy specimens per patient: 1 for PAR staining and another for γ-H2A.X staining. Images were obtained under an inverted fluorescence microscope (BZ-X700, Keyence Corporation, Itasca, Illinois) with 20× objective, which covered the most area of the biopsy specimen in one visual field. Raw imaging data were analyzed using BZ-X analyzer software (Keyence Corporation) to quantify the fluorescence intensity of the PAR signal merged with DAPI in each nucleus. Subsequently, we set the threshold to detect PAR-positive nuclei based on a histogram of the fluorescence intensity and confirmed that each PAR-positive region recognized by the software showed high PAR signal intensity on each section. Supplemental Figure 1 shows an example of the distribution of fluorescence intensity for each nucleus stained with either PAR or γ-H2A.X in either LVRR-positive or LVRR-negative patients. The software automatically calculated the proportion of PAR-positive nuclei (% PAR nuclei) ([PAR stained nuclei] / [all nuclei stained by DAPI]). All raw imaging data were analyzed using

FIGURE 1 Enrollment and F/U of Study Population



DCM = dilated cardiomyopathy; F/U = follow-up; EMB = endomyocardial biopsy; LVRR = left ventricular reverse remodeling; PAR = poly(ADP-ribose).

the same algorithm. The same analysis was also performed for the immunostaining of γ-H2A.X. To analyze the types of PAR-positive cells, 2 researchers jointly reviewed all imaging data of PAR-stained cells to determine whether each stained cell was a cardiomyocyte or noncardiomyocyte; determinations were made based on morphological differences. Noncardiomyocytes are very small compared with typical large mature cardiomyocytes, and their nuclei are located close to the cell membrane, which is detected by WGA staining (15,21).

STATISTICAL ANALYSIS. Continuous variables were expressed as the mean ± SD, and categorical variables as count and proportion. For those variables with skewed distributions, median (interquartile range [IQR]) were reported and compared using the Wilcoxon rank sum test. Statistical significance between the 2 groups was determined by an unpaired 2-tailed Student's *t*-test.

Univariable screening of all parameters of the patients at baseline was first performed. Student's *t* test was used for continuous variables. Fisher's exact test was used for categorical variables and mid-p values were calculated. We used the Kaplan-Meier method and the log-rank test to assess the impact of LVRR on the endpoint. We estimated the effect of %PAR as well as %γ-H2A.X for the combined endpoint by inverse probability weighted Cox proportional hazards regression models with robust standard errors. The weight for each subject was calculated using the generalized propensity score, including the following variables in the model to adjust for baseline confounding factors: age, BMI, family history, duration of heart failure, New York Heart Association (NYHA) functional class, systolic blood pressure, B-type natriuretic peptide (BNP), left ventricular end-diastolic diameter, severe mitral regurgitation (grade III or IV). The associations between %PAR and LVRR as well as between %γ-H2A.X and LVRR were also examined using the propensity score method in logistic regression modeling (22). Receiver-operating characteristic (ROC) analysis was performed to assess the performance of %PAR nuclei as well as %γ-H2A.X to predict LVRR. Cutpoint analysis was performed to determine the optimal cutoff value to maximize the Youden index [sensitivity − (1 − specificity)]. Areas under the ROC curve were calculated using logistic regression.

All analyses were carried out using SAS software version 9.4 (SAS Institute, Inc., Cary, North Carolina). For all tests, a probability value of $p < 0.05$ was considered significant.

RESULTS

CHARACTERISTICS OF THE STUDY POPULATION. A total of 82 patients underwent endomyocardial biopsy and were diagnosed with DCM during the study period. Among these, 24 (29.9%) patients were excluded mainly because they had already received full administration of optimal medical therapy with

high doses of β-blockers at the time of endomyocardial biopsy. As a result, we enrolled 58 (70.1%) patients in this study (Figure 1). The clinical characteristics and laboratory data at the time of biopsy for both LVRR-positive and LVRR-negative groups are presented in Table 1. The patients were predominantly men. The mean age of patients at diagnosis was 44.3 ± 15.0 years. A family history of DCM was identified in 25.9% of all cases. More than one-half of the patients belonged to NYHA functional class III or IV at baseline (58.6%), with a severe reduction of left ventricular systolic function (LVEF 24.2 ± 9.7%). The patients in the LVRR-negative group had significantly lower blood pressure, BMI, and peak oxygen consumption levels. They had a longer QRS duration and more complete left bundle branch block on electrocardiography; larger left ventricular dimensions, with severe mitral regurgitation in echocardiography; and higher BNP levels. However, analyses of these parameters showed no statistically significant differences.

TABLE 1 Baseline Characteristics of the Patients

	LVRR-Negative Group (n = 33)	LVRR-Positive Group (n = 25)	p Value
Age, yrs	45.3 ± 15.4	42.9 ± 14.6	0.558
Male	25 (75.8)	21 (84.0)	0.394
BMI, kg/m^2	21.6 ± 2.9	24.3 ± 5.5	0.019
Smoking	13 (39.4)	10 (40.0)	0.691
Familial DCM	12 (36.4)	3 (12.0)	0.054
Duration of HF, days	231 (101–1,108)	67 (33–107)	0.001
NYHA functional class III, IV	18 (54.5)	16 (44.0)	0.603
SBP, mm Hg	96.0 ± 14.6	111.1 ± 21.1	0.002
DBP, mm Hg	60.7 ± 12.8	70.9 ± 18.6	0.017
HR, beats/min	78.7 ± 19.1	81.6 ± 18.8	0.568
CLBBB	7 (21.2)	4 (16.0)	0.623
Atrial fibrillation	7 (21.2)	3 (12.0)	0.396
QRS duration, ms	115 (108–160)	110 (98–120)	0.085
LAD, mm	43.5 ± 9.4	43.9 ± 8.6	0.857
IVS, mm	7.9 ± 1.8	8.5 ± 1.6	0.214
LVPW, mm	8.4 ± 1.6	9.0 ± 1.7	0.132
LVDd, mm	68.1 ± 9.4	66.1 ± 11.3	0.472
LVDs, mm	60.9 ± 10.8	58.8 ± 11.7	0.465
LVEF (%)	24.5 ± 10.8	23.8 ± 8.1	0.795
MR severity grade			0.447
0 (no MR)	4 (12.1)	6 (24.0)	
I, II	23 (69.7)	16 (64.0)	
III, IV	6 (18.2)	3 (12.0)	
Peak Vo_2, ml/min/kg	15.8 ± 4.3	21.3 ± 3.4	0.002
Hb, g/dl	14.1 ± 2.1	14.9 ± 1.9	0.130
Alb, g/dl	4 ± 0.5	3.9 ± 0.6	0.575
Cr, mg/dl	1 ± 0.2	0.9 ± 0.2	0.428
eGFR, ml/min/1.73 m^2	68.7 ± 19.2	75.3 ± 19.4	0.204
Na, mEq/l	133.9 ± 17.7	139.7 ± 1.8	0.109
BNP, pg/ml	435.9 (203.6–844.0)	348.3 (153.7–617.7)	0.367
ACE inhibitor	16 (48.5)	10 (40.0)	0.513
ARB	9 (27.3)	5 (20.0)	0.454
β-blocker	23 (69.7)	11 (44.0)	0.082
Antimineralocorticoids	20 (60.6)	10 (40.0)	0.152
Loop diuretics	20 (60.6)	12 (48.0)	0.340
Anticoagulant agents	18 (54.5)	7 (28.0)	0.048
ICD implantation	3 (9.1)	0 (0)	0.163
CRT-D implantation	3 (9.1)	0 (0)	0.163

Values are mean ± SD, n (%), or median (interquartile range).

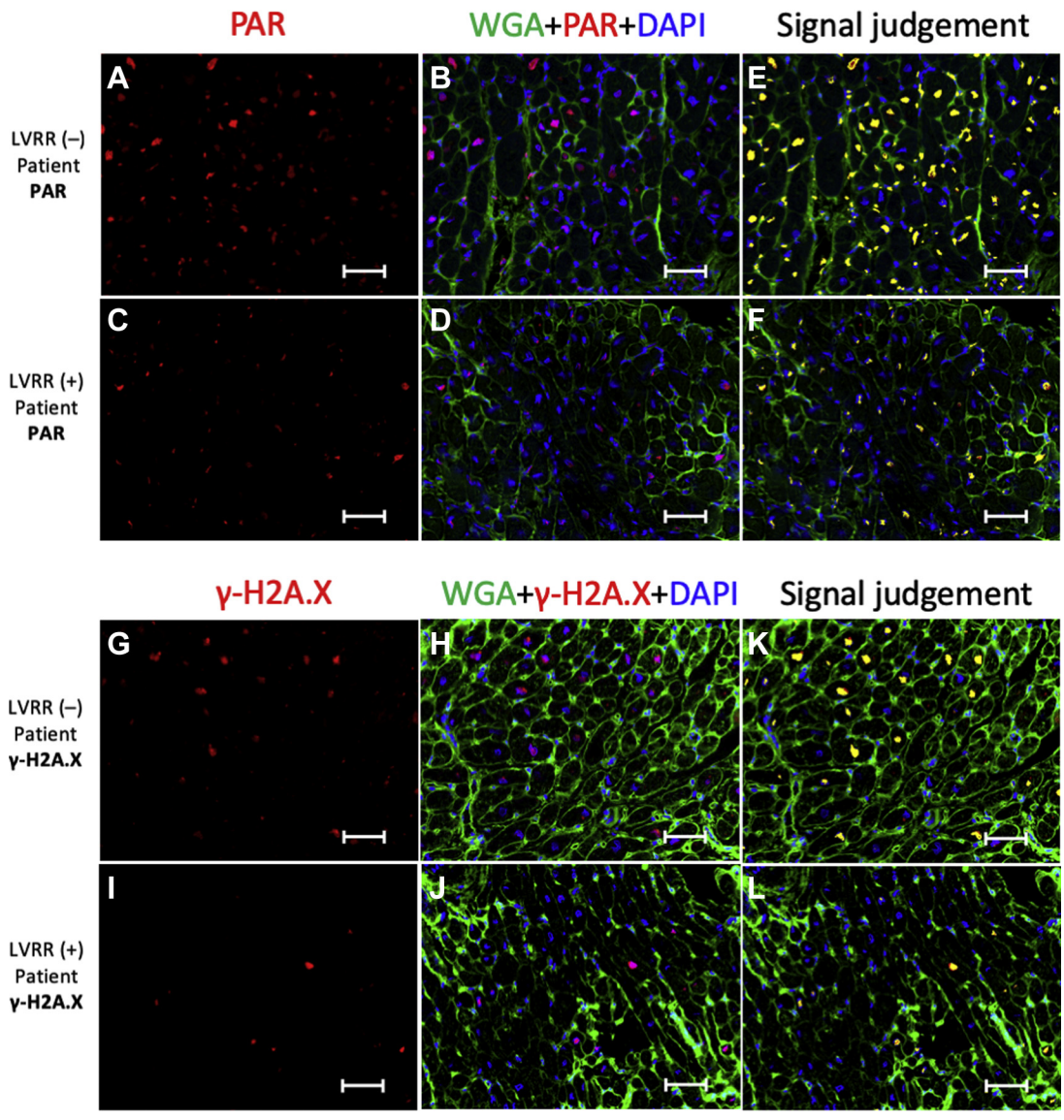
ACE = angiotensin-converting enzyme; Alb = albumin; ARB = angiotensin II receptor blocker; BNP = B-type natriuretic peptide; BMI = body mass index; CLBBB = complete left bundle branch block; Cr = creatinine; CRT-D = cardiac resynchronization therapy with defibrillator; DBP = diastolic blood pressure; DCM = dilated cardiomyopathy; eGFR = estimated glomerular filtration rate; Hb = hemoglobin; HF = heart failure; HR = heart rate; ICD = implantable cardioverter-defibrillator; IVS = interventricular septum; LAD = left atrial diameter; LVDd = left ventricular end-diastolic diameter; LVDs = left ventricular end-systolic diameter; LVEF = left ventricular ejection fraction; LVPW = left ventricular posterior wall; LVRR = left ventricular reverse remodeling; MR = mitral regurgitation; NYHA = New York Heart Association; SBP = systolic blood pressure.

PAR STAINING OF MYOCARDIAL BIOPSY SPECIMENS. Figure 2 shows an example of PAR and γ-H2A.X staining and the analysis via imaging software. Figures 2A to 2D are raw images of immunofluorescence staining for PAR using an endomyocardial biopsy specimen from LVRR-negative and LVRR-positive patients, respectively. Figures 2E and 2F are the same image after automatic assessment by a hybrid cell counting program; PAR-positive nuclei recognized by the program were marked as yellow. Figures 2G to 2L are raw images and program-processed images for γ-H2A.X. Supplemental Figure 1 shows the distribution of fluorescence intensity for each nucleus stained with either PAR or γ-H2A.X measured by the image software. Generally, both PAR and γ-H2A.X were stained in each nucleus (Supplemental Figure 2A). Nuclei with positivity to DNA damage markers were mainly thought to belong to cardiomyocytes. All PAR-positive noncardiomyocytes belonged to the cardiac fibroblast population (Supplemental Figures 2B and 2C). The average proportions of cardiomyocytes and noncardiomyocytes among all PAR-positive cells of all biopsy specimens from 58 patients (1,068 cells) were 94.5% and 5.5%, respectively (Supplemental Figure 2D).

As each biopsy specimen contained different numbers of cells, the numbers of the nuclei varied. The mean numbers of the analyzed nuclei of each PAR-stained specimen in LVRR-negative and LVRR-positive groups were 887 ± 41 and 903 ± 69, respectively (p = 0.832) (Figure 3A). Measurements of PAR staining revealed that patients with LVRR had significantly lower %PAR nuclei (3.7% [IQR: 0.6% to 3.9%] vs. 16.3% [IQR: 6.3% to 19.3%]; p < 0.001) as well as %γ-H2A.X nuclei (3.5% [IQR: 1.2% to 6.4%] vs. 11.7% [IQR: 6.0% to 14.6%]; p < 0.001) compared with those without LVRR (Figures 3B and 3C).

PATIENT OUTCOMES. The median observation period was 1,386 (IQR: 667 to 2,032) days in our study. During the judgement period of LVRR, 25 of 58 (43.1%) patients had achieved LVRR after multidisciplinary

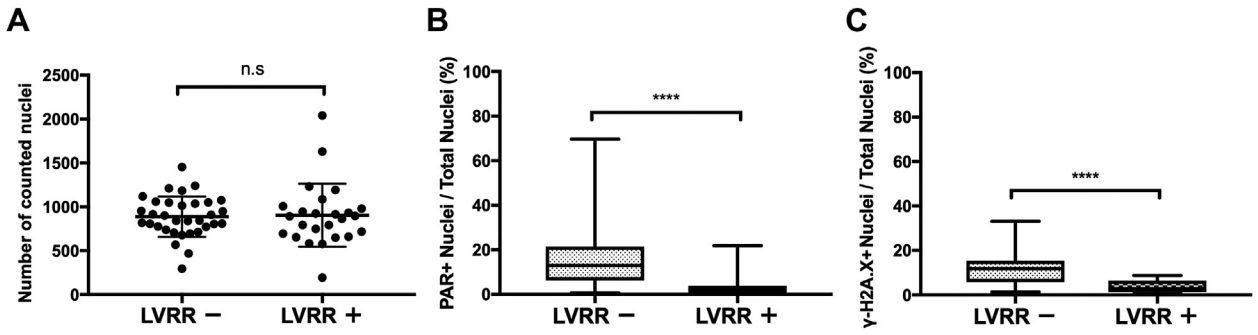
FIGURE 2 Example of Analysis of PAR and γ-H2A.X Staining



(A to D) Raw images of immunofluorescence staining for PAR **(red)** and wheat germ agglutinin (WGA) **(green)** with 4,6-diamidino-2-phenylindole (DAPI) **(blue)** nuclear counterstain using an endomyocardial biopsy specimen (**white bar** = 50 μm) from LVRR-negative and LVRR-positive patients, respectively. **(E, F)** The same image after automatic judgment by hybrid cell count program; PAR-positive nuclei were marked **(yellow)**. **(G to J)** Raw image of immunofluorescence staining for γ-H2A.X **(red)** and WGA **(green)** DAPI **(blue)** nuclear counterstain using an endomyocardial biopsy specimen (**white bar** = 50 μm) from LVRR-negative and LVRR-positive patients, respectively. **(K, L)** The same image after automatic judgment by a hybrid cell count program; γ-H2A.X-positive nuclei were marked **(yellow)**. Abbreviations as in Figure 1.

therapy including inhibition of the renin-angiotensin-aldosterone system, β-blockers, and cardiac resynchronization therapy. Neurohormonal drug treatment with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, antimineralocorticoids, and β-blockers was tailored in the majority of patients (95%, 78%, and 100%, respectively) at target doses. No significant differences were noted between patients with LVRR and those without LVRR with respect to drug administration proportions of angiotensin-converting enzyme inhibitors (68.0% in patients with LVRR vs. 72.7% in patients without LVRR; $p = 0.775$), angiotensin II receptor blockers (28.0% vs. 21.2%; $p = 0.758$), antimineralocorticoids (72.0% vs. 81.8%;

FIGURE 3 Comparison of Analyzed Nuclei and Percentages of PAR-Positive and γ-H2A.X-Positive Nuclei in LVRR-Negative and LVRR-Positive Groups



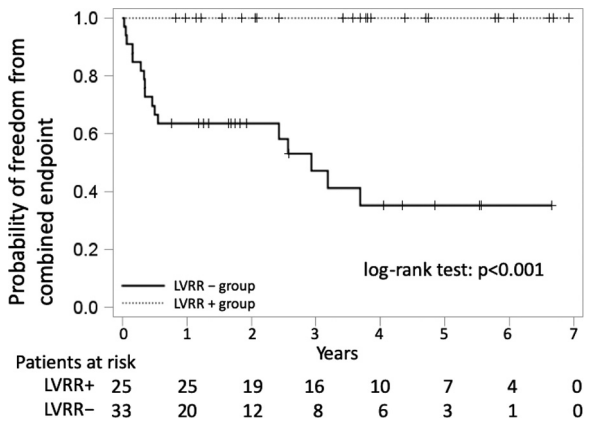
(A) Comparison of the number of counted nuclei on each specimen in the LVRR-negative and LVRR-positive groups. **(B, C)** Comparison of the proportion of PAR-positive or g-H2A.X positive nuclei in the LVRR-negative and LVRR-positive groups, respectively. Bar graph shows the mean proportion of PAR-positive or g-H2A.X-positive nuclei with SDs. ****$p < 0.001$. Abbreviations as in Figure 1.

$p = 0.527$), or β-blockers (both groups achieved 100% administration and the daily doses were 22.7 mg vs. 19.5 mg as equivalents of carvedilol; $p = 0.281$) at 12 months after the initiation of optimal medical therapy. The survival curves of the study population classified according to the presence or absence of LVRR are shown in Figure 4. The patients with LVRR had significantly better prognosis compared with those without LVRR (log-rank test, $p < 0.001$). The combined endpoint was reached during the follow-up period in 17 patients: 8 heart transplants (13.8%), 16 LVAD implants (27.6%), and 1 death (1.7%).

In this study, we used age, BMI, family history, duration of heart failure, NYHA functional classification, systolic blood pressure, BNP, left ventricular end-diastolic diameter, and severe mitral regurgitation (grade III or IV) as confounders for adjustment in propensity score analysis. Propensity score analysis for combined endpoint revealed that %PAR nuclei (for every 10% increase, hazard ratio: 1.36; 95% confidence interval [CI]: 1.02 to 1.81; $p = 0.035$) was a significant and independent prognostic factor after adjustment for other major clinical factors (Table 2). Other major prognostic factors predicting poor outcome in univariable Cox analysis were age, high NYHA functional class, low blood pressure, and low BMI. Because only a small number of patients in our cohort had undergone cardiac magnetic resonance (27.6%) and cardiopulmonary exercise testing (51.7%), we could not include late gadolinium enhancement extent and peak oxygen consumption in the model of propensity score analysis. The %PAR nuclei (for every 1% increase, odds ratio: 0.87; 95% CI: 0.79 to 0.95; $p = 0.003$) was also identified by propensity score analysis as a significant independent predictor of LVRR (Table 3). The results of the same analysis using the data for γ-H2A.X staining are shown in Tables 2 and 3, which revealed that %γ-H2A.X-stained nuclei can also independently predict LVRR.

Figure 5 shows the results of the ROC analysis. Compared with γ-H2A.X staining, PAR staining showed an incremental prognostic power for LVRR, but the statistical difference between these 2 models was not significant ($p = 1.000$). The ideal cutoff value of %PAR nuclei to predict LVRR in our study cohort was 5.74%. Using this threshold, the %PAR nuclei predicted LVRR with a sensitivity of 77.8% (95% CI: 57.7% to 91.4%) and a specificity of 87.1% (95% CI: 70.2% to 96.4%), and the area under the ROC curve was 0.879.

FIGURE 4 Long-Term Prognostic Impact of LVRR in DCM Patients



Survival curves of long-term freedom from major adverse cardiac events (death, ventricular assist device implantation, and heart transplantation) comparing the LVRR-positive and LVRR-negative patient subgroups. Abbreviations as in Figure 1.

TABLE 2 Univariable Cox Analysis and Propensity Score Analysis for Combined Endpoint

	HR (95% CI)	p Value
Age (10-yr increase)	0.66 (0.48–1.49)	0.015
BMI (5-yr increase)	0.46 (0.23–3.21)	0.028
Family history	2.21 (0.84–5.83)	0.110
Duration of HF (per 12-month increase)	1.07 (0.97–1.20)	0.190
NYHA functional class (1 increase in grade)	2.14 (1.14–1.88)	0.018
SBP (10-mm Hg increase)	0.50 (0.34–1.98)	<0.001
BNP (10-pg/ml increase)	1.00 (0.99–1.03)	0.730
LVDd (10-mm increase)	1.13 (0.71–1.44)	0.620
Severe MR (III/IV)	1.48 (0.43–5.17)	0.536
%PAR nuclei (10% increase)	1.25 (0.96–1.63)	0.100
%γ-H2A.X nuclei (10% increase)	1.16 (0.55–2.44)	0.700
Inverse probability weighting using propensity score		
%PAR nuclei (10% increase)	1.36 (1.02–1.81)	0.035
%γ-H2A.X nuclei (10% increase)	1.28 (0.75–2.21)	0.370

CI = confidence interval; HR = hazard ratio; PAR = poly(ADP-ribose); other abbreviations as in Table 1.

DISCUSSION

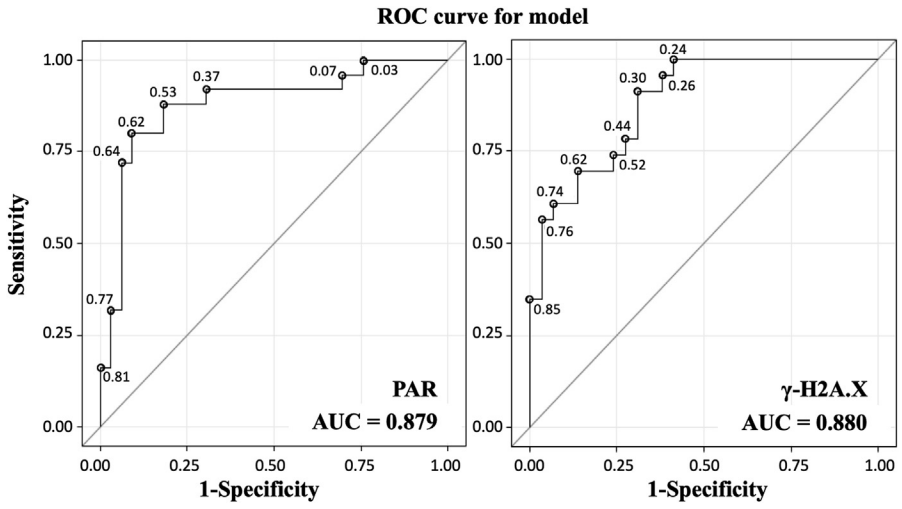
There is consensus that a distinct subset of patients with DCM can achieve LVRR after medical therapy, and that their clinical prognosis is better than those without LVRR (2,3). Over many years, several clinical trials have been conducted to evaluate potential predictors of cardiac recovery in patients with DCM. These trials were generally divided into 2 categories. The first category was the measurement of parameters associated with left ventricular dysfunction such as left ventricular end-diastolic diameter, QRS duration or complete left bundle branch block, and BNP. The second category was the evaluation of myocardial fibrosis assessed by late gadolinium enhancement in cardiac magnetic resonance. Compared with these examinations, the role of endomyocardial biopsy has been very limited. endomyocardial biopsy is mainly used to rule out diseases that are similar to DCM, such as myocarditis and sarcoidosis. Previous reports have established a relationship between quantitative histological findings on endomyocardial biopsy and left ventricular contractile function in patients with DCM (23). In recent years, however, the value of histopathological findings correlated with clinical and hemodynamic parameters has become controversial or even unfavorable (9,24). Therefore, one could question the clinical merit and rationale of assessing myocardial histology by endomyocardial biopsy given the risk of various complications (25). However, late gadolinium enhancement measured by cardiac magnetic resonance provides limited information on interstitial fibrosis, and echocardiography can estimate only myocardial status by compiling information on dysfunction and loss of cardiomyocytes. In contrast, endomyocardial biopsy analysis has the potential to directly assess all of the molecular characteristics of cardiac cells. In this study, we demonstrated the utility of immunostaining DNA damage markers using endomyocardial biopsy specimens to predict the probability of LVRR and even the patient's outcome. Among the analyzed specimens, the predictive ability of PAR for prognoses (combined endpoint and LVRR) is slightly better than that of γ-H2A.X. A previous study involving a small number of patients with heart failure (not limited to DCM) showed that mechanical unloading by LVAD implantation reduced DNA damage responses (26). Taken together with our results, DNA damage seems to be a very important pathophysiological component of heart failure. DCM-causative genes encode a heterogeneous group of molecules that participate in force generation, force transmission, sarcomere integrity, and cytoskeletal and nuclear architecture (27). We have previously reported the prognostic value of genetic mutations in Japanese patients with DCM (28). At first glance, it looks like the majority of DCM-causative genes such as sarcomere and cytoskeletal genes have no relationship with DNA damage. However, the linker of the nucleoskeleton and cytoskeleton complex physically couples the nuclear membrane with the cytoskeleton (29). Therefore, sarcomere and cytoskeletal impairment may also have a harmful impact on the nucleus. Although we do not know the genetic mutations among our study population, the significantly high proportions of PAR-positive nuclei seen in poor-prognosis patients suggests that the accumulation of DNA damage is a common pathogenetic trait in severe

TABLE 3 Univariable Logistic Regression Analysis and Propensity Score Analysis for LVRR

	OR (95% CI)	p Value
Age (10-yr increase)	0.90 (0.63–1.28)	0.551
BMI (5-yr increase)	2.20 (1.08–4.46)	0.029
Family history	0.24 (0.06–0.97)	0.045
Duration of HF (per 12-month increase)	0.72 (0.52–0.99)	0.044
NYHA functional class (1 increase in grade)	1.18 (0.63–2.21)	0.610
SBP (10-mm Hg increase)	1.62 (1.15–2.28)	0.006
BNP (10-pg/ml increase)	0.99 (0.98–1.00)	0.260
LVDd (10-mm increase)	0.82 (0.49–1.39)	0.460
Severe MR (III/IV)	0.61 (0.14–2.74)	0.522
%PAR nuclei (1% increase)	0.81 (0.71–0.92)	<0.001
%γ-H2A.X nuclei (1% increase)	0.68 (0.55–0.84)	<0.001
Inverse probability weighting using propensity score		
%PAR nuclei (10% increase)	0.87 (0.79–0.95)	0.003
%γ-H2A.X nuclei (10% increase)	0.68 (0.55–0.84)	<0.001

OR = odds ratio; other abbreviations as in Tables 1 and 2.

FIGURE 5 ROC Curve to Evaluate the Performance of %PAR or %γ-H2A.X Nuclei to Predict LVRR



AUC = area under the curve; ROC = receiver-operating characteristic; other abbreviations as in Figure 1.

heart failure patients with DCM. Although familial DCM patients were much more common in the LVRR-negative group than in the LVRR-positive group, Tables 2 and 3 show %PAR could independently predict combined endpoint and LVRR even after adjustment for various factors, including family history. In fact, LVRR-negative patients showed high %PAR in both the familial and nonfamilial DCM groups (Supplemental Figures 3A and 3B). To our knowledge, this is the first clinical study to show that the evaluation of DNA damage is useful for the prediction of prognosis in patients with heart failure. Considering the high specificity and sensitivity, this kind of evaluation of DNA damage may also prove useful in patients with heart failure from etiologies other than DCM.

STUDY LIMITATIONS. Despite the significance of the findings, our study has several limitations. First, because our hospital conducts most of the heart transplants in Japan, the majority of patients with DCM who are referred to our hospital are at end-stage heart failure, and are treated with LVAD implantation or heart transplantation. Therefore, our study population imposes a selection bias with respect to the characteristics of DCM among the general population. For many patients referred to our hospital, some pharmacological medications such as renin-angiotensin-aldosterone system inhibitors and β-blockers have already been administered in the referring hospital, even before a diagnosis of DCM has been confirmed. Although we excluded patients who had already completed full administration and up-titration of medications for heart failure at the time of endomyocardial biopsy, the remaining patients still had an incomplete course of medication, as shown in Table 1. Thus, low-dose drugs might have had a small impact on DNA damage in the patients with heart failure. This is an unavoidable consequence of a retrospective study conducted in a large referral center. We need to prospectively enroll patients who have not received any pharmacological agents for heart failure.

Second, the sample size in our study population is small and, as mentioned before, we could not include the extent of late gadolinium enhancement or peak oxygen consumption in the propensity score analysis, which makes it impossible to compare %PAR nuclei with these gold standards for assessing prognosis. Third, although we paid careful attention to the immunofluorescence staining of the endomyocardial biopsy specimens, this process requires effort and time. Besides, the results of immunostaining will be affected by many factors such as the quality of antibodies, the storage conditions of endomyocardial biopsy specimens, and the technical skills of the staff. Therefore, simpler methods to measure DNA damage should be developed.

CONCLUSIONS

In this study, we demonstrated the utility of PAR staining to predict the probability of LVRR. Our

study underscores the importance of utilizing endomyocardial biopsy specimens to evaluate DNA damage at baseline and to improve the prognostic stratification in patients with DCM. Further prospective multicenter studies are needed to assess the prognostic significance of PAR staining and to establish the most effective strategies for the diagnosis of DCM and identify patients who have a poor prognosis. Optimal treatment could then be initiated earlier, thereby improving patient outcome.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: DNA damage has been reported to cause heart failure in animal models. This was the first study that clearly showed the existence of DNA damage in human cardiomyopathy heart tissues and the correlation between degree of DNA damage and clinical prognosis. Staining of PAR, a pathogenic signature of DNA damage responses, is useful to anticipate the probability of left ventricular reverse remodeling and prognosis in patients with DCM.

TRANSLATIONAL OUTLOOK: Further prospective multicenter studies are needed to assess the clinical usefulness of PAR staining for predicting the prognosis among heart failure patients.

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KEY WORDS dilated cardiomyopathy, DNA damage, left ventricular reverse remodeling, poly ADP-ribose

APPENDIX For supplemental figures, please see the online version of this paper.