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Speckle-Tracking 2-Dimensional Strain Echocardiography: A New Noninvasive Imaging Tool to Evaluate Acute Rejection in Cardiac Transplantation

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Abstract

Background—There remains no reliable noninvasive method to detect cardiac transplant rejection. Recently, speckle-tracking 2-dimensional strain echocardiography was shown to be sensitive in early detection of myocardial dysfunction in various models of cardiomyopathy. We aim to determine if 2DSE derived functional indices can detect cardiac transplant rejection.

Methods—Heterotopic rat cardiac transplantation was performed in histocompatible, isografts (ISO) or histoincompatible allografts (ALLO). Histological rejection scores were determined. Short-axis, mid-left ventricular (LV) echocardiography was performed on post-transplant day 6. Conventional measures of function such as LV fractional shortening and ejection fraction as well as 2-dimensional strain echocardiography parameters were measured.

Results—Despite class IIIB rejection in ALLO and no rejection in ISO, there was no difference in fractional shortening or ejection fraction (ISO vs. ALLO: 15±3 vs. 12±3%; 36±5 vs. 26±6%, respectively, both not significant). In contrast, 2DSE revealed decreases in global radial strain (ISO vs. ALLO: 12.6±5.6 vs. 1.1±0.2 %, P<0.05), peak radial systolic strain rate (ISO vs. ALLO: 3.10±0.74 vs. 0.54±0.13 s⁻¹, P<0.001) and peak circumferential systolic strain rate (ISO vs. ALLO: -1.99±0.55 vs. -0.43±0.11 s⁻¹, P<0.01).

Conclusions—Systolic strain imaging using 2-dimensional strain echocardiography differentiates myocardial function between experimental cardiac transplant rejection in ALLO and non-rejection in ISO. 2-dimensional strain echocardiography may be useful in early noninvasive detection of transplant rejection.

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Introduction

Cardiac transplant rejection is associated with adverse prognosis if untreated. Currently, rejection in cardiac transplants is diagnosed by right-ventricular endomyocardial biopsy which is considered the 'gold standard' method for identifying cardiac transplant rejection. Endomyocardial biopsy, an invasive procedure for the ongoing surveillance of cardiac rejection, is usually performed several times over the first year post-transplantation. It is associated with potential complications such as tricuspid valve damage, cardiac perforation and arrhythmias.^{1,2} More recently it was demonstrated that the development of tricuspid regurgitation correlated with the number of endomyocardial biopsies.³ Alternative noninvasive techniques for detection of cardiac transplant rejection such as echocardiography, nuclear imaging⁴ and magnetic resonance imaging^{5–8} have been tested. However, no reliable alternative to endomyocardial biopsy has emerged. Thus, there continues to be an ongoing search for new methods and technologies that could be used as a noninvasive alternative.

Echocardiography is a noninvasive technique that had shown early promise in correlating functional changes associated with endomyocardial biopsy-proven rejection in human cardiac transplant recipients.⁹ However, conventional echo methods using M-mode or 2-dimensional echocardiography such as fractional shortening or ejection fraction are limited to assessing changes in ventricular cavity size during the cardiac cycle. Furthermore, results of a more recent large population of heart transplant patients revealed that no single parameter or combination of parameters emerged as sufficiently able to eliminate surveillance endomyocardial biopsy to detect cardiac rejection.¹⁰

The traditional cavity measurement-based echocardiographic parameters enumerated above are indirect means of assessing myocardial function and for which our group has found to be insensitive to early changes in cardiomyopathy. In contrast speckle tracking echocardiography that directly assesses myocardial mechanics was more sensitive and accurate in the early detection of doxorubicin or ischemic cardiomyopathy than conventional echocardiographic measure of systolic dysfunction such as fractional shortening and ejection fraction.^{11,12} Similarly strain rate imaging but not conventional echocardiography was found to detect LV dysfunction in aged mice.¹³ Strain represents deformation, an intrinsic mechanical property and is a more direct measure of systolic function¹² versus conventional cavity-based echocardiographic measures.

Diagnosis of acute sub-clinical rejection in cardiac transplant recipients is important. Given that some patients may be asymptomatic despite histological evidence of rejection, it is important to consider the potential that the newer speckle-tracking technology might be more useful than conventional echocardiography to determine rejection. We hypothesized in the present study that 2-dimensional strain echocardiography can detect allo-immune induced LV dysfunction in experimental cardiac transplantation under conditions of histological rejection.

Methods

Transplantation

All animal procedures were approved by the local institutional animal care and use committee. All animals received humane care in compliance with the "Principles of Laboratory and Animal Care" and the "Guide for the Care and Use of Laboratory Animals". Lewis (Lew:RT1^l) and Wistar-Furth (WF:RT1^u) rat strains (Harlan Labs, Indianapolis, IN) were chosen for genetic disparity at both the major and minor histocompatibility loci for donor-to-recipient combination of Lewis→Lewis (for ISO) or Wistar-Furth→Lewis (for

ALLO) rats. Isogeneic (n=6) or allogeneic (n=8), heterotopic cardiac transplantation was performed aseptically in pentobarbital (50 mg/kg, i.p.) anesthetized animals as earlier described in our laboratory.¹⁴ Donor hearts were arrested in ice-cold University of Wisconsin preservation solution and transplanted into anesthetized (50 mg/kg pentobarbital i.p.) recipient animals.

Histological rejection scoring

Tissue was fixed in 10% phosphate-buffered formalin. Paraffin-embedded sections were stained with hematoxylin and eosin. Histological rejection was scored blinded using criteria established by the International Society for Heart and Lung Transplantation (ISHLT).

Echocardiography

Echocardiography was performed on post-transplant day 6 using a Vivid 7 echocardiograph (General Electric, Waukesha WI) and a 11 MHz M12L linear array transducer. Standard parasternal short-axis mid LV view was used with the papillary muscles as anatomic landmarks. Image depth was 2–2.5 cm, frame rates of 234–256 frames per second and second harmonic imaging were used. The images were sent to a separate workstation for analysis using EchoPAC with Q analysis software (General Electric, Waukesha WI). Echocardiographic analysis was performed by investigators blinded to the experimental group.

LV size (internal diameter in diastole, LVIDd and systole, LVIDs) and thickness (anterior and inferior diastolic thickness, At and It respectively) were measured using anatomical M-mode of the 2-dimensional B-mode images through the plane of the anterior and inferior segments. Fractional shortening (FS) was calculated using the formula $FS = (LVIDd - LVIDs) / LVIDd \times 100\%$. LV ejection fraction was calculated using the Teicholz method.¹⁵ LV mass was derived using the formula: $0.8[1.04\{(It + LVID + At)^3 - LVID^3\}] + 0.6$.¹⁶ Diastolic measurements were performed at the R wave of electrocardiographic gating that corresponded to the donor heart rhythm in cases where two distinct R waves were seen (i.e., from the native and heterotopically-transplanted donor heart). In cases where the donor heart rhythm could not be distinguished from the native heart on electrocardiography, diastolic measurements were done at the largest diameter of the heart during the cardiac cycle.

Radial and circumferential strain and strain rate were measured as previously published.¹¹ In brief, the mid-portion of the LV was divided into 6 segments as defined by the American Society of Echocardiography.¹⁷ The endocardial border was traced while the outer border was adjusted to fit the epicardial contour. The software automatically selected stable acoustic objects within the myocardium to track and compute strain and strain rate in radial and circumferential directions in the segments of the mid-ventricle throughout the cardiac cycle. Peak systolic and peak early diastolic strain and strain rate were obtained for each segment and the average used to compute global radial or circumferential strain and strain rates.

Data and Statistical analysis

Data are expressed as means $\pm$ standard error of means. Statistical analysis was performed using unpaired Student's *t* test or Mann Whitney U test, where appropriate, with significance set at $p < 0.05$.

Results

Histological evidence of grade IIIB rejection in rat heart ALLO at post-transplant day 6 was evidenced by the presence of inflammatory cell infiltrate, myocyte necrosis, interstitial

edema, hemorrhage and vasculitis (Figure 1). None of these features (i.e. grade 0) were exhibited in ISO controls or in native hearts of either ISO or ALLO recipients. Graded scoring revealed a significant increase in rejection over ISO controls or native hearts of ALLO recipients.

Echocardiography indicated that LV mass was significantly ($P<0.0006$) elevated in rat ALLO compared to rat ISO controls (Figure 2). LV ejection fraction, fractional shortening, internal diameter in systole and end-systolic volume were not significantly different between ALLO and ISO controls (Figure 3).

An example of radial strain is shown throughout the cardiac cycle in an ALLO vs. ISO controls (Figure 4). Global radial strain in ALLO was significantly ($P<0.05$) decreased from that seen in ISO controls (Figure 5 A). Global circumferential strain showed a similar trend (Figure 5 B). However, this was not statistically significant.

Decreases in both peak systolic and peak early diastolic radial strain rate were seen in ALLO compared to ISO controls (example shown in Figure 6). Quantitatively, global peak systolic and peak early diastolic radial strain rates were decreased in ALLO compared to ISO controls (Figure 7 A and B). Similarly, global peak systolic and peak early diastolic circumferential strain rate were significantly ($P<0.01$ and $P<0.001$, respectively) decreased in ALLO vs. ISO controls (Figure 7 C and D).

Discussion

In the present study, we examined the potential usefulness of speckle tracking-based 2-dimensional strain echocardiography in the evaluation of cardiac transplant rejection in an experimental rat transplant model. A major finding in this study is that radial strain, peak systolic radial and circumferential strain rate and peak early diastolic radial and circumferential strain rate are reduced in cardiac transplants undergoing allo-immune rejection vs. transplants without rejection. Secondly, these strain-based measures detected LV dysfunction during histological rejection at a time when conventional echocardiographic measures such as fractional shortening or ejection fraction were not able to detect differences in systolic function between the two groups. Our new findings in an experimental cardiac transplant model are consistent with prior studies showing 2-dimensional strain echocardiography can detect LV dysfunction in a variety of experimental and clinical settings under asymptomatic conditions that could not be detected using conventional systolic echocardiographic measures of LV function.^{18,19} The value of 2-dimensional strain echocardiography arises from its properties to directly measure wall deformation which is an intrinsic mechanical property versus conventional cavity-based echocardiographic analysis of LV systolic function.

The findings in the present study confirm the traditional finding that LV mass is increased during allo-immune rejection in experimental models²⁰ and in human^{20–22} cardiac transplant rejection. This increased LV mass has been attributed acutely to inflammatory cell infiltration and graft edema.²² Chronically, the increase in LV mass in cardiac transplants has been attributed to hypertrophy arising from various factors including repetitive rejection episodes, immunosuppression-induced hypertension therapy, chronic tachycardia and denervation.²²

The concept of using strain imaging as a tool to evaluate cardiac rejection is new. In a study published in 2009, tissue Doppler imaging was able to show for the first time a correlation between systolic radial strain rate in the anterior wall and rejection grade but no correlation with ejection fraction during chronic rejection in a heterotopic rat cardiac transplant model although ejection fraction was clearly decreased at all grades of histological rejection.²³ This

was followed by another study using a different technique of magnetic resonance strain imaging that showed decreased circumferential strain despite normal ejection fraction in a rat heart-lung transplant model.²⁴ Our results using 2-dimensional strain imaging of global strain determined over 6 segments of LV wall in a model of acute rejection are consistent with these recent findings. Collectively the MRI study and our study indicate that strain imaging discriminates dysfunction independent of loading conditions and that strain imaging may be a useful tool for acute and chronic rejection. In addition we expand on this promising approach by showing that both systolic and diastolic radial and circumferential strain rate as well as radial strain are all decreased in allo-immune-induced cardiac transplant rejection. Although strain and strain rate can be measured by both Doppler as well as tissue speckle-tracking techniques, the Doppler method has the disadvantage of being angle-dependent, potentially limiting its versatility.¹⁹

Furthermore, our study demonstrates differences not only in systolic strain but also in early diastolic strain rate between ALLO and ISO hearts. Additional evaluation of diastolic dysfunction by strain and strain imaging is potentially important clinically since diastolic dysfunction as evidenced by tissue Doppler echocardiography frequently occurs before development of systolic dysfunction in cardiac transplant patients.²⁵

Limitations of the Study

For technical reasons, analysis of longitudinal strain and strain rate imaging is difficult in the rat model of cardiac transplantation. Due to mechanical unloading as a result of heterotopic cardiac transplantation in rodents, the echocardiographic values of conventional systolic function and strain are typically lower than normal hearts. Because of this, comparisons to pre-operative strain measurements of donor hearts or native hearts of graft recipients were deemed to be invalid for purposes of functional analysis. To circumvent this limitation, we used time-matched, heterotopic isograft controls for the allo-immune rejection group. Furthermore because of the short time-frame of acute rejection in this rat strain model, we avoided serial echocardiographic measurements which would necessitate inhalation anesthetic in order to eliminate any modulation of graft function. Inhalation anesthetics are well known to produce preconditioning or cardioprotection via increased NO synthesis that could directly impact not only myocardial function but also immune function and complicate interpretation of the study. Our study also did not examine a detailed correlation between gradations of histological rejection score and strain and strain rate. Rather, the present paper was designed specifically to illustrate the potential of the new 2-dimensional strain echocardiography technique to discriminate LV dysfunction in rejecting vs. non-rejecting cardiac transplants under conditions in which global echocardiographic markers of systolic dysfunction were not detected. In future studies, careful registration between regional strain and histological rejection will allow a more definitive correlative analysis between these two measures.

Because abnormal strain can occur in multiple pathologies,^{11,12,26} it is a nonspecific finding that may not necessarily arise from transplant rejection. Therefore, the potential clinical use is as a screening tool to identify transplant patients that would require endomyocardial biopsy for confirmation of pathology, but strain echocardiography would not supplant the need for biopsy. However, despite this nonspecificity, the potential clinical utility remains undiminished with strain echocardiography possibly being useful for screening of patients needing biopsy, instead of current practice of needing invasive biopsy as a screening method.

Strain imaging and clinical cardiac rejection

It is acknowledged that findings in this rodent model of heterotopic cardiac transplantation may not necessarily be extrapolated to the clinical situation. Nevertheless the findings here provide the experimental basis for systematically validating the potential use of 2-dimensional strain echocardiography in evaluating human cardiac transplant rejection as there are no published reports to our knowledge of the use of this new technology in human cardiac transplant recipients. In this context using a Doppler strain imaging technology, a recent published report has emerged demonstrating systolic strain dysfunction in a small population of human heart transplant recipients with ISHLT rejection grade $\geq 1B$ but not $\leq 1B$.²⁷ Authors found systolic strain dysfunction occurring without differences in conventional echocardiographic analysis of ejection fraction, wall thickness and other parameters.

Despite the promise of the clinical study using Doppler strain imaging, the study had limitations. The study examined only systolic strain/strain rate and only in a single posterior wall segment of the LV which may limit its discriminating efficacy due to heterogeneity in histological rejection. Another significant concern is that there were no baseline values provided. Thus there was no way of discerning whether variances in early post-transplant myocardial function existed which may have arisen due to differences in initial donor heart performance or to differences in donor heart ischemic times which may have impacted post-transplant performance and echocardiographic values and altered the course of rejection and the later echocardiographic measurements of strain. Finally, this clinical study was limited to the evaluation of systolic longitudinal and radial strain and strain rate. Collectively, the present study and the recent clinical study indicate the potential promise of utilizing 2-dimensional strain echocardiography as a new tool in diagnosis of cardiac transplant rejection.

Advantages of speckle-tracking imaging over current imaging techniques

Tissue Doppler-based strain imaging is severely limited by its angle-dependency and being affected by translational motion. Therefore this technique can only estimate strain along the ultrasound beam. Tissue Doppler imaging requires the use of high frame rates in contrast to lower frame rates for 2-dimensional strain imaging. It has been acknowledged that there is less artifact with the newer 2-dimensional strain imaging and considerably less intra-observer and inter-observer variability with 2-dimensional strain imaging than for Doppler-based strain imaging.¹⁹ The far less expensive cost of 2-dimensional strain imaging over techniques such as magnetic resonance is an attractive feature. Finally, the advancement of sophisticated algorithms has allowed the data analysis for 2-dimensional strain imaging to be fairly rapid allowing a much more timely acquisition of data compared to current tissue Doppler-based strain imaging analysis.

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Figure 1.
Example showing normal histology in rat cardiac isograft but not allograft. Histological rejection in allograft was indicated by multi-focal inflammatory cell infiltrate, myocyte necrosis, hemorrhage and vasculitis.

Figure 2. Echocardiographic evidence of increased LV mass in allografts (n=8) compared to isograft controls (n=6). ***P<0.001

Figure 3. M-mode echocardiography revealed no significant changes in systolic function (fractional shortening, ejection fraction, LV internal diameter in systole, end-systolic volume) in allografts compared to isograft controls.

Figure 4.
Example images showing qualitative decreases in radial strain in allograft (lower panel) vs. isograft (upper panel) control.

Figure 5.
Decreased global radial (* $P < 0.05$) and circumferential (not significant, $P = 0.108$ by Mann Whitney U test) strain in allografts vs. isograft controls.

Figure 6.
Example images showing peak systolic and diastolic radial strain rate in allograft (lower panel) and isograft (upper panel) control hearts.

Figure 7. Decreased global systolic and diastolic circumferential strain rate and global systolic and diastolic radial strain rate in allografts vs. isograft controls. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$