Mitral Leaflet Changes Following Myocardial Infarction
Clinical Evidence for Maladaptive Valvular Remodeling

Jonathan Beaudoin, MD; Jacob P. Dal-Bianco, MD; Elena Aikawa, MD, PhD; Joyce Bischoff, PhD; J. Luis Guerrero, BS; Suzanne Sullivan, BS; Philipp Emanuel Bartko, MD; Mark D. Handschumacher, BS; Dae-Hee Kim, MD, PhD; Jill Wylie-Sears, MS; Jacob Aaron, BS; Robert A. Levine, MD

Background—Ischemic mitral regurgitation (MR) is classically ascribed to functional restriction of normal leaflets, but recent studies have suggested post–myocardial infarction (MI) mitral valve (MV) leaflet fibrosis and thickening, challenging valve normality. Progression of leaflet thickness post-MI has not been studied. We hypothesized that excessive MV remodeling post-MI contributes to MR. Our objectives are to characterize MV changes after MI and relate them to MR.

Methods and Results—Three groups of 40 patients with serial echocardiograms over a mean of 23.4 months were identified from an echocardiography database: patients first studied early (6±12 days) and late (12±7 years) after an inferior MI and normal controls. MV thickness was correlated with MR. We studied the mechanisms for MV changes in a sheep model (6 apical MI versus 6 controls) followed for 8 weeks, with MV cellular and histopathologic analyses. Early post-MI, leaflet thickness was found to be similar to controls (2.6±0.5 vs 2.5±0.4 mm; P=0.23) but significantly increased over time (2.5±0.4 to 2.9±0.4 mm; P<0.01). In this group, patients tolerating maximal doses of renin–angiotensin blocking agents had less thickening (25% of patients; P<0.01). The late-MI group had increased thickness (3.2±0.5 vs 2.5±0.4 mm; P<0.01) without progression. At follow-up, 48% of post-MI patients had more than mild MR. Increased thickness was independently associated with MR. Experimentally, 8 weeks post-MI, MVs were 2-fold thicker than controls, with increased collagen, profibrotic transforming growth factor-β, and endothelial-to-mesenchymal transformation, confirmed by flow cytometry.

Conclusions—MV thickness increases post-MI and correlates with MR, suggesting an organic component to ischemic MR. MV fibrotic remodeling can indicate directions for future therapy. (Circ Cardiovasc Imaging. 2017;10:e006512. DOI: 10.1161/CIRCIMAGING.117.006512.)

Key Words: ischemic mitral regurgitation  mitral valve  myocardial infarction  valvular disease

Ischemic mitral regurgitation (MR) is a frequent complication of myocardial infarction (MI), doubling heart failure and mortality. Its primary mechanism is leaflet tethering by disturbed left ventricle (LV) geometry secondary to local or global remodeling. Ischemic MR is considered functional, without contribution from intrinsic leaflet changes. The size of the valve was classically assumed to be fixed in the remodeling ventricle; however, the mitral valve (MV) can enlarge to adapt to ventricular expansion and reduce MR. Experimental studies have demonstrated that mechanical stretch induced by leaflet tethering can induce active valve growth. This compensatory mechanism, however, is frequently unable to compensate for LV distortion, and ischemic MR remains prevalent.

See Editorial by Watanabe See Clinical Perspective

Other work suggested that abnormal leaflets could contribute to functional MR: increased valve stiffness and collagen accumulation were observed in end-stage heart failure, suggesting organic MV alteration. Based on collagen synthesis upregulation in response to stress, Kunzelman et al showed by finite-element analysis that increased leaflet stiffness and thickness can interfere with MV function. Normal closure requires flexible leaflets to bend and form a coaptational seal, and normal MV can expand 15% in systole to close (Figure 1); these elements of normal function could be limited with stiffer leaflets. Mechanisms and clinical implications of these fibrotic changes have not been investigated. Especially, it is unclear if and how initially compensatory stretch-induced valve growth can progress toward maladaptive stiffening and thickening contributing to MR. In the setting of MI, numerous processes such as renin–angiotensin system activation are known to affect LV remodeling; but whether leaflet tissue can be involved in the remodeling process is unknown.

We tested the hypothesis that post-MI MV changes could result in maladaptive fibrosis, with excessive thickening...
contributing to MR. The prevalence and timing of valve thickening and their relation to MR were assessed. We studied over time 3 groups of patients: those with recent acute MI, those with remote MI, and normal controls. In a subsidiary study, we used a sheep model of MI to explore cellular and molecular mechanisms leading to fibrosis. To capture changes caused by MI without the papillary muscle displacement seen with infarcted posterior MI or the associated MR, which can secondarily thicken the leaflets, we created limited antero-apical MIs that do not generate MR.

### Methods

#### Human Retrospective Follow-Up Study

We used our institution’s echocardiography database to identify patients with documented MI involving at least the inferior or posterior walls, leaflet tethering, and at least 2 echocardiograms separated by ≥90 days (Figure 2). The first 40 consecutive patients were studied in each of the 3 groups: (1) patients with an echo within the first 3 weeks of a first MI and a follow-up study at least 90 days later (early-MI group); (2) patients with remote (>5 years) MI and comparable follow-up (late-MI group); and (3) control subjects (comparable age and sex to both MI groups) with similar follow-up to control for changes in leaflet thickness with aging. The control group consisted of patients with serial normal echoes either after foramen ovale closure or screening for chemotherapy-induced cardiomyopathy. Exclusion criteria were more than mild aortic stenosis or insufficiency, MV organic pathology (prolapse, rheumatic, endocarditis, or extensive annular calcification), valve prosthesis, Marfan disease, and patient taking anti-parkinsonian dopamine agonists.

#### Echocardiographic Analysis

All echocardiograms were reviewed blinded to the patient group and timing of the examination (Figure 3). MV thickness was measured in parasternal long-axis and apical zoomed views in a diastolic frame without valve motion, with leaflets perpendicular to the echocardiographic beam, taking advantage of the axial resolution. This was achieved at mid-diastole in parasternal view and end-diastole in apical views. Thickness was measured 3× for both leaflets in both views in areas free of chordal attachment; all values were averaged. A subset of 10 patients was measured by 2 investigators and 1 month later by the first investigator for interobserver and intraobserver variabilities. MV excursion angle between end-systole and full opening was measured in the parasternal long-axis view, LV end-diastolic and end-systolic dimensions were measured and LV ejection fraction (EF) calculated (modified Quinones method). MR was graded independently by 2 level-3 readers (discrepancies were resolved by a third reader as needed) as suggested by current guidelines with an integrated approach using all available parameters. MR grade was defined as: 1 (no or trace), 2 (mild: effective regurgitant orifice <20 mm²; regurgitant volume <30 mL; vena contracta, <0.3 cm), 3 (moderate: effective regurgitant orifice, 0.20–0.39 mm²; regurgitant volume, 30–59 mL; vena contracta, 0.3–0.69 cm), or 4 (severe: effective regurgitant orifice >40 mm²; regurgitant volume, ≥60 mL; vena contracta, ≥0.7 cm). In this population, more than mild MR (as defined by effective regurgitant orifice >20 mm² or regurgitant volume >30 mL) has been shown to have a prognostic impact. For that reason, greater than mild MR was considered significant in our study. Progression of MR was defined as an increase of at least one grade of MR between baseline and follow-up studies. Medical records were reviewed for clinical characteristics and medication profile. The study was approved by the institutional review committee.

#### Experimental Study

Six adult Dorsett hybrid sheep underwent left anterior descending artery ligation, and 6 had sham thoracotomy. Epicardial echocardiography was done at baseline and euthanasia. MVs were harvested at 8 weeks for analyses. Thickness (averaged over 10 thickest midleaflet sites), valve morphology, and collagen accumulation were analyzed by Hematoxylin/Eosin and Masson’s trichrome staining. Cellular activation (endothelial-to-mesenchymal transformation [EMT]) was assessed by staining for endothelial (CD31) and interstitial myofibroblast (α-smooth muscle actin [α-SMA]) markers. EMT was confirmed by flow cytometry of dissociated fresh valve endothelial cells with fluorescent anti-CD31 and anti-α-SMA labeling. Transforming growth factor (TGF-β) was assessed by immunohistochemistry as an initial measure of growth signaling promoting both EMT and fibrosis.

#### Statistics

Continuous variable were expressed as mean ± standard deviation, and categorical variables as number (%). Differences in means were tested for significance with Student’s t tests and differences in proportions with χ² tests. Differences in thickness over time among groups were assessed with repeated-measures analysis of variance and paired t test. Variables associated with more than mild MR for all post-MI patients at follow-up were assessed by logistic regression. Leaflet thickness, LV EF, LV end-diastolic dimension, LV end-systolic dimension, age, leaflet excursion, time from infarct date, tethering distances (from papillary muscles to annulus), left atrial dimension, and annulus dimensions (apical 2- and 4-chamber views) in mid-systole were tested in univariate analysis, and variables with P<0.05 were tested in multivariable model. Interobserver and intraobserver agreements of leaflet thickness measurements were assessed using a single measure, 2-way random effect intraclass correlation coefficient. Values of 0.893 (interobserver) and 0.946 (intraobserver) were obtained. Correlation and Bland–Altman plots are presented in Figure I in the Data Supplement. In the animal model, EMT by flow cytometry and echocardiographic measures were compared between MI and control groups with t tests. Statistical analysis was performed with Stata/IC 11.2 (StataCorp LP, TX).

#### Results

#### Human Studies

A total of 120 patients were studied, each with 2 echocardiographic studies. The first echocardiogram was performed...
3 Beaudoin et al Mitral Valve Fibrosis After Myocardial Infarction

6±12 days post-MI (85% within 1 week) in the early-MI group and 12±7 years post-MI in the late-MI group. There was no significant difference in age, sex, or median follow-up time (642, 544, and 773 days) among groups. Both MI groups had lower LVEF and larger LV dimensions than normal group (Table 1). Most patients had revascularization at the time of MI (early-MI, 33/40; late-MI, 36/40). Primary PCI was the preferred revascularization strategy for both groups (70% and 60% for early-MI and late-MI groups). Reasons for nonrevascularization included late presentation without ongoing chest pain, absence of reversible ischemia on noninvasive testing, or patients refusing invasive procedures.

Mitral Valve Thickness and Motion
Average MV thickness was stable over time in the control group (2.6±0.5 to 2.6±0.5 mm; \( P=0.71 \); Figure 4). The early-MI group had initially similar thickness to controls (2.5±0.4 versus 2.6±0.5 mm; \( P=0.23 \)) but showed significant progression over time (2.5±0.4 to 2.9±0.4 mm; \( P<0.001 \)). The proportion of early-MI patients with thickness >3 mm increased from 13% at baseline to 43% at follow-up (\( P<0.01 \); see example in Figure 3 and Movie I through III in the Data Supplement). Late-MI patients had thicker leaflets compared with early-MI and control patients at baseline (3.2±0.5 versus 2.5±0.4 versus 2.6±0.5 mm for late-MI, early-MI, and controls; \( P<0.01 \)). The proportion of patients with increased thickness was higher in the late-MI group (68%, 13%, and 18% of patients had thickness >3 mm in late-MI, early-MI, and control groups; \( P<0.01 \)). Despite increased thickness at baseline, the late-MI group had no progression over time. Opening excursion for both leaflets was stable over time in the control group. At baseline, the early-MI group had decreased excursion compared with controls, and there was an additional significant decrease at follow-up (Table 2 and Figure 3). The late-MI group had reduced leaflet excursion at baseline compared with control and early-MI groups but was stable over time. There was no significant difference in leaflet thickness for patients with versus without revascularization or post-MI ischemia by noninvasive tests.

Figure 3. Representative examples showing mitral leaflet evolution after myocardial infarction (MI), thickness, and leaflet excursion measurements. A and B, Immediately after MI, parasternal and apical views showing a thin valve with full diastolic excursion. C and D, Same patient 5 years later. The mitral leaflets are thicker, with limited excursion. The systolic function and ventricular dimensions did not change between the 2 examinations. E, Another patient with initially thin and mobile valve (Movie I in the Data Supplement) with definite thickening, reduced excursion, and severe mitral regurgitation (MR) 3 years later (F and G; Movies II and III in the Data Supplement). Displayed frames show the maximal diastolic leaflet opening.
Beaudoin et al Mitral Valve Fibrosis After Myocardial Infarction

There was no observed difference in thickness for patients with associated comorbidities (diabetes mellitus, renal failure, hypertension, hyperlipemia, or active smoking). No difference in thickness was observed based on the echocardiography system used (51% Philips ie-33; 31% Philips Sonos 7500; 18% GE Vivid 7).

Effect of Medication on Thickness

Medication profile is shown in Table 1: 75% of late-MI and 93% of early-MI patients were treated with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (Figure 5). However, the tolerated dose was low (<50% of maximal dose for a given medication) for a significant proportion of patients (66/80). In an exploratory analysis, we tested the hypothesis that leaflet thickness progression could be different in patients taking higher doses of angiotensin-converting enzyme inhibitor or angiotensin receptor blockers. In the subgroup of early-MI patients taking higher doses, there was no significant leaflet thickening (high doses: 2.6±0.4 to 2.7±0.6 mm, P=0.44; low doses: 2.5±0.4 to 2.9±0.4 mm, P<0.01). In the late-MI group, anterior leaflets were thinner in the high-dose subgroup (anterior thickness: 2.9±0.6 versus 3.4±0.5 mm; P=0.04) However, our study was not designed and had limited power to assess this difference.

Association of Leaflet Thickness and Ischemic MR

In the pooled ensemble of post-MI patients, those with increase of at least 1 grade of MR at follow-up versus baseline had also significant increase in average leaflet thickness (+0.43±0.46 versus +0.06±0.46 mm, for patients with versus without MR progression; P=0.002; Figure 5). At follow-up, there was a significant association between leaflet thickness and greater than mild MR (average thickness 3.2±0.5 versus 2.8±0.4, P=0.0006, by guest on November 29, 2017 http://circimaging.ahajournals.org/ Downloaded from

---

Table 1. Control, Late MI, and Early MI Groups Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (n=40)</th>
<th>Early MI (n=40)</th>
<th>Late MI (n=40)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time post-MI</td>
<td>...</td>
<td>6±12 days</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>66±13</td>
<td>66±11</td>
<td>70±10</td>
<td>0.17</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>26 (65)</td>
<td>25 (63)</td>
<td>30 (75)</td>
<td>0.45</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>69±7</td>
<td>38±10*</td>
<td>41±11*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>46±6</td>
<td>49±6</td>
<td>55±7*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>29±5</td>
<td>37±8*</td>
<td>44±7*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tethering distance: PPM, mm</td>
<td>38±5</td>
<td>41±5</td>
<td>42±5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tethering distance: LPM, mm</td>
<td>37±5</td>
<td>40±5</td>
<td>43±6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MR=mild</td>
<td>0 (0)</td>
<td>13 (33)*</td>
<td>19 (48)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Anterior thickness, mm</td>
<td>2.7±0.6</td>
<td>2.7±0.5</td>
<td>3.4±0.6*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Posterior thickness, mm</td>
<td>2.5±0.5</td>
<td>2.3±0.5</td>
<td>3.0±0.5*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Average thickness, mm</td>
<td>2.6±0.5</td>
<td>2.5±0.4</td>
<td>3.2±0.6*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Excursion anterior leaflet, degree</td>
<td>64±14</td>
<td>41±15*</td>
<td>38±14*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Excursion posterior leaflet, degree</td>
<td>62±19</td>
<td>47±17*</td>
<td>38±14*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>23 (58%)</td>
<td>27 (68%)</td>
<td>31 (78%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2 (5%)</td>
<td>10 (25%)*</td>
<td>14 (35%)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>19 (48%)</td>
<td>25 (63%)*</td>
<td>35 (88%)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>2 (5%)</td>
<td>6 (15%)*</td>
<td>15 (38%)†</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>20 (50%)</td>
<td>37 (93%)*</td>
<td>30 (75%)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ß-Blockers</td>
<td>15 (38%)</td>
<td>38 (98%)*</td>
<td>36 (90%)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aspirin</td>
<td>24 (60%)</td>
<td>37 (93%)*</td>
<td>34 (85%)*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Statin</td>
<td>19 (48%)</td>
<td>37 (93%)*</td>
<td>36 (90%)*</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

ACEI indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; LPM, lateral papillary muscle; LVEDD, left ventricle end-diastolic diameter; LVEF, left ventricle ejection fraction; LVESD, left ventricle end-systolic diameter; MI, myocardial infarction; MR, mitral regurgitation; and PPM, posterior papillary muscle.

*P<0.05 vs control.
†P<0.05 vs early MI.

---

Figure 4. Leaflet thickness at 2 time points in control, early myocardial infarction (MI), and late MI groups. Early MI group shows significant progression over time. Late MI group presents increased but stable thickness. *P<0.01 vs control and early MI (baseline and follow-up); †P<0.01 vs early MI baseline.

Figure 5. Leaflet thickness at 2 time points in control, early myocardial infarction (MI), and late MI groups. Early MI group shows significant progression over time. Late MI group presents increased but stable thickness. *P<0.01 vs control and early MI (baseline and follow-up); †P<0.01 vs early MI baseline.

---

Table 2. Early MI Population at Baseline and Follow-Up

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-Up</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days post-MI</td>
<td>6±12</td>
<td>77±3558</td>
<td></td>
</tr>
<tr>
<td>LVEF, %</td>
<td>38±10</td>
<td>38±13</td>
<td>0.83</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>49±6</td>
<td>53±7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>37±8</td>
<td>42±8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tethering distance: PPM, mm</td>
<td>41±5</td>
<td>42±5</td>
<td>0.13</td>
</tr>
<tr>
<td>Tethering distance: LPM, mm</td>
<td>40±5</td>
<td>41±4</td>
<td>0.23</td>
</tr>
<tr>
<td>Annulus AP4, mm</td>
<td>35±4</td>
<td>36±3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Annulus AP2, mm</td>
<td>35±4</td>
<td>35±3</td>
<td>0.6</td>
</tr>
<tr>
<td>MR&gt;2</td>
<td>13 (33%)</td>
<td>19 (48%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Anterior thickness, mm</td>
<td>2.7±0.5</td>
<td>3±1.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Posterior thickness, mm</td>
<td>2.3±0.5</td>
<td>2.6±0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Average thickness, mm</td>
<td>2.5±0.4</td>
<td>2.9±0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Excursion anterior leaflet, degree</td>
<td>41±15</td>
<td>37±17</td>
<td>0.03</td>
</tr>
<tr>
<td>Excursion posterior leaflet, degree</td>
<td>47±17</td>
<td>40±19</td>
<td>0.01</td>
</tr>
</tbody>
</table>

AP2 indicates apical 2-chamber; AP4, apical 4-chamber; LPM, lateral papillary muscle; LVEDD, left ventricle end-diastolic diameter; LVEF, left ventricle ejection fraction; LVESD, left ventricle end-systolic diameter; MI, myocardial infarction; MR, mitral regurgitation; and PPM, posterior papillary muscle.
for patients with versus without MR), without difference in LVEF ($P=0.36$) or LV dimensions ($P=0.48$ for LV end-diastolic dimension and $0.99$ for LV end-systolic dimension; Table in the Data Supplement). Age ($P=0.003$) and left atrial dimension ($P=0.001$) were significantly associated with MR in univariate analysis. In multivariate analysis, average thickness ($\beta$ coefficient±SE: $1.47±0.62$; $P=0.018$) and age ($\beta$ coefficient±SE: $0.058±0.027$; $P=0.027$) were the factors associated with greater than mild MR, while LA dimension was not ($P=0.85$).

**Experimental Mechanistic Study**

All animals survived until euthanasia; none developed MR. There was a mild decrease in LVEF post-MI ($61±7\%$ to $47±4\%$; $P<0.01$). MV thickness (microscopy) was significantly increased post-MI versus sham ($1.02±0.23$ versus $0.44±0.11$ mm; $P<0.01$). Histopathology post-MI showed expansion of the central spongiosa layer and focal subendothelial deposition of collagen, primarily on the atrial leaflet surface (Figure 6, arrows).

**Cellular Changes**

The endothelial layer of control MVs had CD31$^+$ cells without $\alpha$-SMA staining (Figure 7). In contrast, post-MI MVs were positive for both CD31 and $\alpha$-SMA, indicating EMT. By flow cytometry, endothelial cells coexpressing $\alpha$-SMA were more common in post-MI versus sham MVs ($48±14\%$ versus $7±4\%$ of endothelial cells; $P<0.01$). The endothelium and subendothelial interstitium were strongly positive for TGF-$\beta$1, colocalized to regions of $\alpha$-SMA staining.

**Discussion**

In this study, we demonstrate that MV presents echocardiographic changes post-MI: (1) Early post-MI, leaflet thickness is initially normal but increases over time; (2) Late post-MI, thickness is

**Figure 5.** Top. Thickness progression early post-myocardial infarction (MI) in patients taking high vs low angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB) doses. Bottom, Post-MI mitral valve (MV) thickness according to the presence of mitral regurgitation (MR).

**Figure 6.** Mitral valves thickness (microscopy) in sham (A) and post-MI (B) sheep. Post-MI valves (C) are significantly thicker ($P<0.01$).
maximal without progression. Although aging is associated with valve thickening, lack of increase in a control group of comparable age, sex, and follow-up time indicates that this is not a likely explanation for the changes observed early post-MI.

The capacity for MV remodeling in functional MR has been demonstrated previously. While active valve expansion can be seen as adaptive, excessive remodeling can lead to fibrosis with increased thickness, decreased mobility, and potentially more MR because effective closure requires systolic expansion and flexibility. Although differences between the late-MI and control groups could be related to other comorbidities, the evolution of changes in the early-MI cohort and the large-animal study are consistent, with changes beginning only after MI, mainly in the early period, as the late-MI group showed stable thickness.

Mechanistic Considerations
Ischemic MR is the complex result of mechanical stretch in an ischemic environment with subsequent heart failure–related humoral activation. A previous study from our group demonstrated that mechanical stretch alone from papillary muscle displacement causes active valve enlargement. In the experimental portion of the current study, we show that an apical MI without papillary muscle involvement also triggers MV alterations. Importantly, strong TGF-β staining post-MI was not observed in the stretch-only model, suggesting a specific role for the ischemic environment on MV remodeling. While MR itself could contribute to the observed changes through increased turbulence and shear stress, the animal cohort showed a cluster of histological changes in the absence of MR.

Activation of renin–angiotensin system is well known post-MI, and angiotensin II can trigger TGF-β expression in the heart, with subsequent collagen deposition and extracellular matrix remodeling. Our observations suggest that valve leaflets can be involved in the global post-MI remodeling, and increased TGF-β likely plays a role in the observed leaflet changes (Figure 8). Although our clinical study was not primarily designed to assess the effect of medication on MV thickness, the observed relation between angiotensin-converting enzyme inhibitor/angiotensin receptor blockers doses and leaflet thickness post-MI deserves attention because it is consistent with TGF-β involvement: renin–angiotensin system blockade is known to inhibit profibrotic effects of TGF-β in various organs, including blood vessels and myocardium. Our data suggest a possible effect of TGF-β on MV remodeling, which could represent a pharmacological target. Although we cannot directly link MR to these histological changes in this experimental work, these observations warrant further investigations because this could be a first step toward medical approaches targeting leaflet fibrosis to prevent ischemic MR.

Clinical Significance of Increased Leaflet Thickness
This study challenges the concept that ischemic MR is exclusively related to LV remodeling. It is reasonable to suggest that disturbed extracellular matrix can change the mechanical properties of the valve, increasing its stiffness. This can limit MV closure by decreasing systolic expansion, limiting flexible leaflet bending, and potentially decreasing adaptive valve growth as compensatory enlargement is attenuated in these patients. All MI patients had the substrates for...
ischemic MR: decreased ejection fraction, inferoposterior MI, and MV tethering. However, not all of them had significant MR, suggesting other variables in the pathogenesis of MR. Leaflet thickness was significantly associated with MR, and in the evolving early-MI group, increased leaflet thickness was associated with MR progression.

Limitations
The human data are retrospective, and unknown factors not accounted for could have explained differences in thickness between groups, but not likely within the early-MI group over time. MR quantification can change according to loading conditions. Our population was limited to preselected patients (inferior MI and leaflet tethering) more likely to have ischemic MR. Difference in individual follow-up timing, absence of 3D valve metrics and relatively small sample size are other limitations. Noninvasive ways to measure fibrosis and biomechanical properties of cardiac valves are limited, but a finite-element model previously demonstrated that increased thickness can improve coaptation.10 Although leaflet thickness was assessed noninvasively in the clinical study, our protocol (averaging 12 measures/patient) showed good reproducibility and correlated well with previous studies.16,10 leaflet thickening post-MI was confirmed by pathology in the sheep study. Despite small sample size in the animal study, observed differences were consistent and highly significant. Our animal protocol included apical MI without MR (contrasting with the clinical study: inferior MI with high ischemic MR prevalence); this was important to demonstrate that the observed leaflet changes are not limited to inferior MI or MR itself. Interestingly, decreased diastolic excursion paralleled the increase in thickness post-MI. Although this can be attributed to decreased cardiac output or diastolic tethering,40 intrinsic leaflet changes could also explain this phenomenon in part and could be explored in future biomechanical studies.

Conclusion
MV undergo multiple changes post-MI. Excessive valve remodeling can result in maladaptive fibrosis, suggesting an organic component in ischemic MR. The role of TGF-β and renin–angiotensin system to modulate this remodeling merits exploration.

Sources of Funding
This work was supported by American Heart Association (AHA)-Founders Affiliate postdoctoral fellowship grant 10POST4580055 (Dr Beaudoin), grant 07CVDO4 of the Leducq Foundation, Paris, France, for the Leducq Transatlantic MITRAL Network, Fonds de Recherche Santé Québec and Heart and Stroke Foundation of Canada (Dr Beaudoin), and by National Institutes of Health (NIH) grants K24 HL67434 and R01 HL109506 (Drs Levine, Bischoff, and Aikawa) and HL128999.

Disclosures
None.

References
20. Timek TA, Lai DT, Dagum P, Liang D, Daughters GT, Ingels NB Jr, Miller DC. Mitral leaflet remodeling in dilated...


Quick DW, Kunzelman KS, Kneebone JM, Cochran RP. Collagen synthesis is upregulated in mitral valves subjected to altered stress. ASAIO J. 1997;43:181–186.


Beaudoin et al Mitral Valve Fibrosis After Myocardial Infarction

CLINICAL PERSPECTIVE

Ischemic mitral regurgitation is generally considered functional: normal leaflets unable to close properly in a dilated and distorted left ventricle. However, recent experimental work in animal models suggested leaflet abnormalities potentially contributing to functional mitral regurgitation. In this article, we compare the progression of mitral valve thickness in 3 groups of patients: those with (1) recent and (2) remote inferior myocardial infarction and (3) normal controls. We show that progressive mitral valve thickening occurs early after myocardial infarction and is associated with later mitral regurgitation. In an associated animal experiment, we demonstrate mitral valve thickening even after a limited apical myocardial infarction, with evidence of fibrotic remodeling and strong presence of remodeling growth factor-β in the leaflets. This clinical and experimental work suggests an organic component to ischemic mitral regurgitation and suggests mitral leaflet remodeling as a potential therapeutic target.
Mitral Leaflet Changes Following Myocardial Infarction: Clinical Evidence for Maladaptive Valvular Remodeling

Jonathan Beaudoin, Jacob P. Dal-Bianco, Elena Aikawa, Joyce Bischoff, J. Luis Guerrero, Suzanne Sullivan, Philipp Emanuel Bartko, Mark D. Handschumacher, Dae-Hee Kim, Jill Wylie-Sears, Jacob Aaron and Robert A. Levine

_Circ Cardiovasc Imaging_. 2017;10:
doi: 10.1161/CIRCIMAGING.117.006512

_Circulation: Cardiovascular Imaging_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2017 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-9651. Online ISSN: 1942-0080

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circimaging.ahajournals.org/content/10/11/e006512

Data Supplement (unedited) at:
http://circimaging.ahajournals.org/content/suppl/2017/10/16/CIRCIMAGING.117.006512.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Cardiovascular Imaging_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Cardiovascular Imaging_ is online at:
http://circimaging.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

1) Supplemental Methods:

**Detailed experimental protocol:** Six adult Dorsett hybrid sheep (weight > 45 kg) were loaded for 3 days with Amiodarone (200 mg orally/day), then anesthetized (propofol, 1 mg/kg IV), intubated and ventilated at 15 ml/kg with 2% Isoflurane-oxygen adjusted by blood-oxygen and CO2 monitoring. Animals prophylactically received Glycopyrrolate (0.4 mg intravenously), Cephazolin (0.5 gm intravenously) and Amiodarone (150 mg intravenous drip) during surgery, and intravenous 0.9% saline solution as needed. After sterile left thoracotomy, an epicardial echocardiography was performed and the distal left anterior descending coronary artery was ligated, avoiding infero-posterior infarction and potential ischemic mitral regurgitation by increased papillary muscle tethering over time. All animals were followed for 8 weeks and sacrificed after left thoracotomy allowing repeated high-quality epicardial echo imaging. After sacrifice the mitral valve was harvested. These sheep were compared to a group of 6 normal sheep without MI, who underwent a sham surgery. Mitral valve tissue harvesting: In a sterile manner and under irrigation of cooled phosphate buffered saline (PBS), sheep hearts were harvested, mitral valve anterior and posterior leaflets were dissected and divided for histopathology (frozen in OCT compound and stored at -80°C) and cell isolation and flow-cytometry (transported fresh in cooled physiologic collecting medium). These studies conform to NIH guidelines for animal care and have Institutional Animal Care Committee approval.

**Echocardiography:** Echocardiography data were collected epicardially using high-frequency 2D and 3D (S5, X3) probes and a Philips iE33 scanner (Andover, MA). Full data sets were acquired in standardized planes at baseline and before sacrifice. MR presence or absence was assessed by color flow Doppler and vena contracta width. Data were digitally stored for offline analysis using...
Xcelera and QLAB 5.1 (Philips, Andover, MA), and the custom software Omni4D (MD Handschumacher).

**Histopathological analyses:** MV leaflets were dissected and 6µm cryo-sections were cut and stained for overall morphology using hematoxylin and eosin. Leaflet thickness was measured by microscopy in ten thickest areas across the midportion of the leaflets. Masson trichrome staining assessed collagen accumulation. Immunohistochemistry was performed with the avidin-biotin-oxidase method as previously described. Endothelial cells (EC) were identified using anti-CD31 antibody (Santa Cruz Biotechnology). The activated valvular interstitial cell (VIC) phenotype was determined by α-smooth muscle actin ([anti-α-SMA]; clone 1A4; Sigma))55-57. Immunofluorescence double labeling was done to confirm co-expression of CD31 and α-SMA in the same cells. To explore mechanisms of cellular changes, leaflet sections were immunostained with anti-TGF-β1 (R&D Systems) to detect latent and activated protein58,59.

**MV cell analysis:** Valve tissue was minced into 1mm x 1mm pieces and incubated with Cell Dissociation Buffer (Invitrogen), an enzyme-free, EDTA-containing solution developed for flow cytometric analysis, for 4-minutes at 37 C at a specific tissue/volume ratio to obtain a single-cell suspension of endothelial and interstitial cells. Flow cytometry was used to quantify valvular cells transitioning between endothelial and mesenchymal phenotypes. ECs were detected and quantified using murine anti-sheep CD31 antibody conjugated to fluorescein isothiocyanate (FITC, ABD Serotec); endothelial cells (stained positive with the anti-sheep CD31-FITC conjugate) transitioning to a mesenchymal phenotype (EMT) were detected using a murine anti-human α-SMA (clone1A4) conjugated to phycoerythrin (R&D Systems). Activated valvular interstitial cells were CD31-negative, α-SMA positive. Anterior and posterior leaflets were analyzed separately.
### Univariate comparison of patients with vs without significant MR at follow-up

<table>
<thead>
<tr>
<th></th>
<th>Trace/mild MR (n=42)</th>
<th>MR &gt; mild (n=38)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64±10</td>
<td>72±9</td>
<td>0.001</td>
</tr>
<tr>
<td>Atrial Fibrillation, n(%)</td>
<td>13 (31)</td>
<td>14 (37)</td>
<td>0.64</td>
</tr>
<tr>
<td>Hyperlipemia, n(%)</td>
<td>31 (74)</td>
<td>29 (76)</td>
<td>1.0</td>
</tr>
<tr>
<td>Renal Failure, n(%)</td>
<td>12 (29)</td>
<td>9 (24)</td>
<td>0.8</td>
</tr>
<tr>
<td>Smoker, n(%)</td>
<td>12 (29)</td>
<td>6 (16)</td>
<td>0.19</td>
</tr>
<tr>
<td>Hypertension, n(%)</td>
<td>27 (64)</td>
<td>31 (82)</td>
<td>0.13</td>
</tr>
<tr>
<td>Diabetes, n(%)</td>
<td>11 (26)</td>
<td>13 (34)</td>
<td>0.47</td>
</tr>
<tr>
<td>Echocardiographic variables:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF(%)</td>
<td>37±13</td>
<td>39±11</td>
<td>0.36</td>
</tr>
<tr>
<td>LVEDD(mm)</td>
<td>54±7</td>
<td>55±8</td>
<td>0.48</td>
</tr>
<tr>
<td>LVESD(mm)</td>
<td>44±8</td>
<td>44±8</td>
<td>1.0</td>
</tr>
<tr>
<td>Tethering Distance, PPM (mm)</td>
<td>43±4</td>
<td>41±5</td>
<td>0.17</td>
</tr>
<tr>
<td>Tethering distance, LPM (mm)</td>
<td>42±4</td>
<td>42±6</td>
<td>0.67</td>
</tr>
<tr>
<td>Left atrium dimension (mm)</td>
<td>39±6</td>
<td>43±5</td>
<td>0.001</td>
</tr>
<tr>
<td>Average thickness(mm)</td>
<td>2.8±0.4</td>
<td>3.2±0.5</td>
<td>0.0006</td>
</tr>
<tr>
<td>Excursion anterior leaflet(degree)</td>
<td>42±15</td>
<td>36±17</td>
<td>0.08</td>
</tr>
<tr>
<td>Excursion posterior leaflet(degree)</td>
<td>42±17</td>
<td>38±17</td>
<td>0.25</td>
</tr>
<tr>
<td>Annulus AP4(mm)</td>
<td>36±3</td>
<td>36±4</td>
<td>0.94</td>
</tr>
<tr>
<td>Annulus AP2(mm)</td>
<td>35±4</td>
<td>37±3</td>
<td>0.06</td>
</tr>
</tbody>
</table>
3) Supplemental Figures

Supplemental Figure 1

Correlation and Bland-Altman graphs for average thickness interobserver variability.
Dot plot of average thickness of individual patients at baseline and follow-up in the early-MI group. The proportion of early-MI patients with thickness >3 mm increased from 13% at baseline to 43% at follow-up (p<0.01).
Supplemental Figure 3

Bar graphs showing the average thickness difference (follow-up vs baseline) in individual patients (control group: average thickness difference 0.02 ± 0.36 mm; 1/40 patients had >0.5 mm increase in thickness; early-MI group: average thickness difference: +0.35±0.38 mm, 15/40 patients had >0.5 mm increase; late-MI group: average thickness difference -0.01±0.27 mm. 1/40 patients had >0.5 mm increase. In the early-MI group, patients with ≥0.35 mm (median value) increase in thickness had MR progression in 55% (12/22) vs 17% (3/18) for those with <0.35 mm increase in thickness (p=0.02).
4) Legends for Supplemental Videos:

**Video 1:** Apical 4-chamber view showing a thin and mobile mitral valve early post-myocardial infarction. Diastolic opening is preserved.

**Video 2:** Apical 4-chamber view of the same patient 3 years later. The valve is significantly thicker, and diastolic excursion reduced.

**Video 3:** Apical 4-chamber view with color Doppler at follow-up showing severe mitral regurgitation.