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

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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
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 views in a diastolic frame without valve motion, with leaflets perpendicular to the echo-cardiographic beam, taking advantage of the axial resolution. This 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 3x 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 thickness 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 thickening, LVEF, 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) 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...
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.
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, Table 2).
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.](image5.png)

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.](image6.png)

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,36 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.17–19,23,26,27,35 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.26 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.19 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,34,35 the animal cohort showed a cluster of histological changes in the absence of MR.

Activation of renin–angiotensin system is well known post-MI,43 and angiotensin II can trigger TGF-β expression in the heart,44–46 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,47,48 including blood vessels and myocardium.49,50 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.21,26 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.18,21 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 impair coaptation. 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, 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, 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.

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**Disclosures**

None.

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**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 transforming 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.