ESC European Society of Cardiology

European Heart Journal - Cardiovascular Imaging (2022) **00**, 1–9
https://doi.org/10.1093/ehjci/jeac050

ORIGINAL PAPER

Histopathological maladaptive changes in the explanted human mitral leaflets correlate with changes in echocardiographic leaflet morphology and the severity of ischaemic mitral regurgitation

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Received 11 November 2021; editorial decision 27 February 2022; accepted 4 March 2022

Aims Several changes of the mitral valve (MV) morphology have been previously documented in ischaemic mitral regurgitation (IMR) upon macro and microscopic examination. This study aimed to correlate echocardiographic MV thickening with IMR severity and to delineate the histopathological basis of valve thickening from the explanted leaflets.

Methods and results Two hundred and fifty patients were included in the echo-group; of these, 48 patients (19.2%) underwent surgical mitral valve replacement (MVR), including them in the histology-group. By echocardiography, the thickness of the anterior and posterior leaflet was more extensive in moderate to severe IMR, $P < 0.001$. Histology-group: patients were divided into two groups based on the median thickness: those with cusp thickness <0.42 cm in Group 1, and ≥0.42 cm in Group 2. The thickness of the base and cusp was more significant in Group 2, $P < 0.05$ in both. Group 2 biopsies were characterized by involvement of the three leaflet segments, myxoid tissue, and fibrosis deposition. Thicker leaflets were associated with a greater degree of mitral regurgitation (MR), $P < 0.0001$. In the echo-group, a median leaflet thickness of 3.5 mm of the anterior and posterior MV was independently associated with moderate to severe ischaemic MR [odds ratio (OR) 2.88, $P < 0.01$] and (OR 10.8, $P < 0.001$), respectively.

Conclusion In ischaemic MR, the thicker the cusps, the worse the MR. Leaflet thickening was due to the myxoid and fibrosis deposition and was detected by echocardiography. Therefore, this method can be helpful in the evaluation of valve remodelling.

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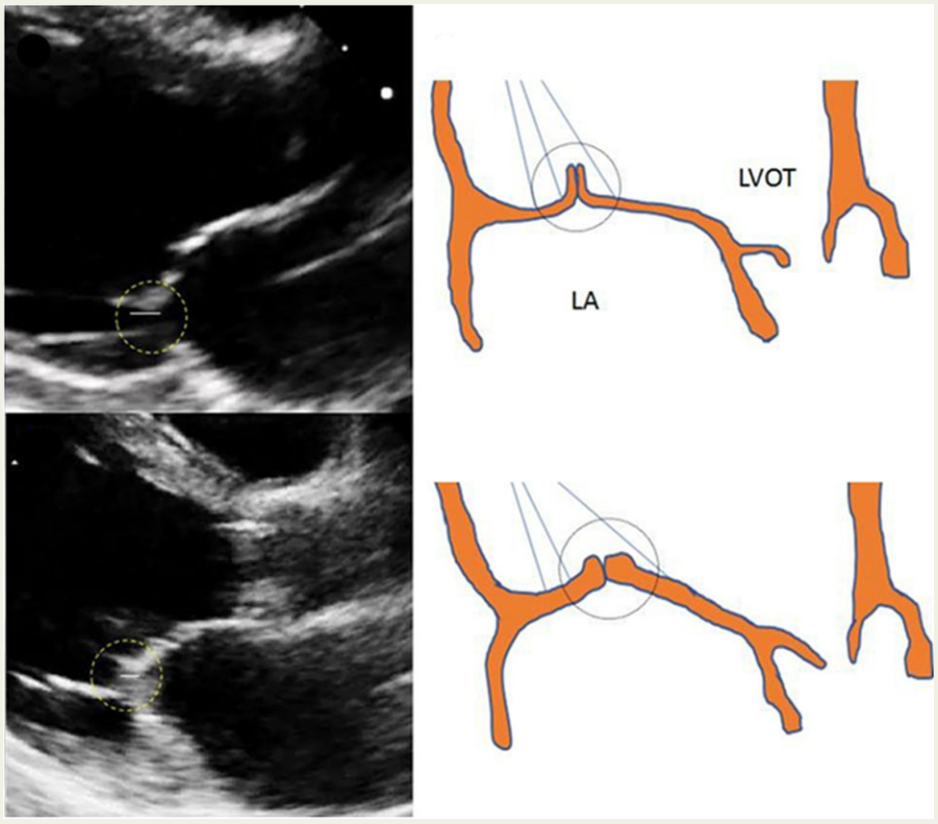
Graphical Abstract



In the pathological remodelling of IMI, the thickness of the cusps of the leaflets is associated with poor coaptation and an increase in the degree of insufficiency. LA, left atrium; LVOT, left ventricular outflow tract.

Keywords ischaemic mitral regurgitation • echocardiography • leaflet stiffness and thickness • histopathology

Introduction

For several years, ischaemic mitral regurgitation (IMR) was described as a consequence of post-infarction left ventricular remodelling, proposing that the retrograde flow occurs in the presence of a structurally normal mitral valve (MV) apparatus.[1] However, growing evidence suggests that the MV undergoes numerous cellular adaptative mechanisms, inducing morphological valvular impairment.[2,3] Although it is known that valvular stress causes greater remodelling with plasticity and/or thickening,[4–6] it remains not well determined whether this maladaptation affects the entire apparatus, the underlying histopathological characteristics of MV thickening, and its association with IMR severity. Therefore, this study correlated echocardiographic MV leaflet thickening with IMR severity; in addition, histological analysis of explanted leaflets from patients with significant IMR helped describe histological features at sites of thickening.

Methods

A retrospective cross-sectional study was conducted between January 2017 and December 2019 at the National Institute of Cardiology 'Ignacio Chavez', in Mexico City. Inclusion criteria for participating in the study were patients older than 18, diagnosed with chronic inferior wall myocardial infarction (IMI) by echocardiography performed at the institution and/or history of IMI documented in the institution's electronic or physical medical records. Chronic infarction was defined as an IMI event ≥6 months before inclusion in the study. Only patients with significant right coronary artery disease (RCA) who underwent percutaneous coronary revascularization (PCI) procedure were included.

Patients were excluded if they had significant coronary lesions in other coronary territories, another moderate to severe valvular disease other than IMR, global left ventricular (LV) remodelling (sphericity index ≤1.5), poor acoustic windows, hypothyroidism, autoimmune disease, infiltrative disease, previous radio, or chemotherapy to the thorax, and those with primary structural abnormalities of the MV (myxomatous, sequelae of rheumatic fever, vegetations, perforation, etcetera). This study followed the Helsinki guidelines and was exempted by the Ethics Committee of our institution due to its retrospective and observational setting.

Echocardiography and measurements

All transthoracic echocardiograms (TTE) performed with Philips iE33 or EPIC ultrasound systems (Philips Healthcare, Andover, MA), and GE vivid 9-XD Clear (GE Healthcare, Waukesha, WI) were evaluated. A standard

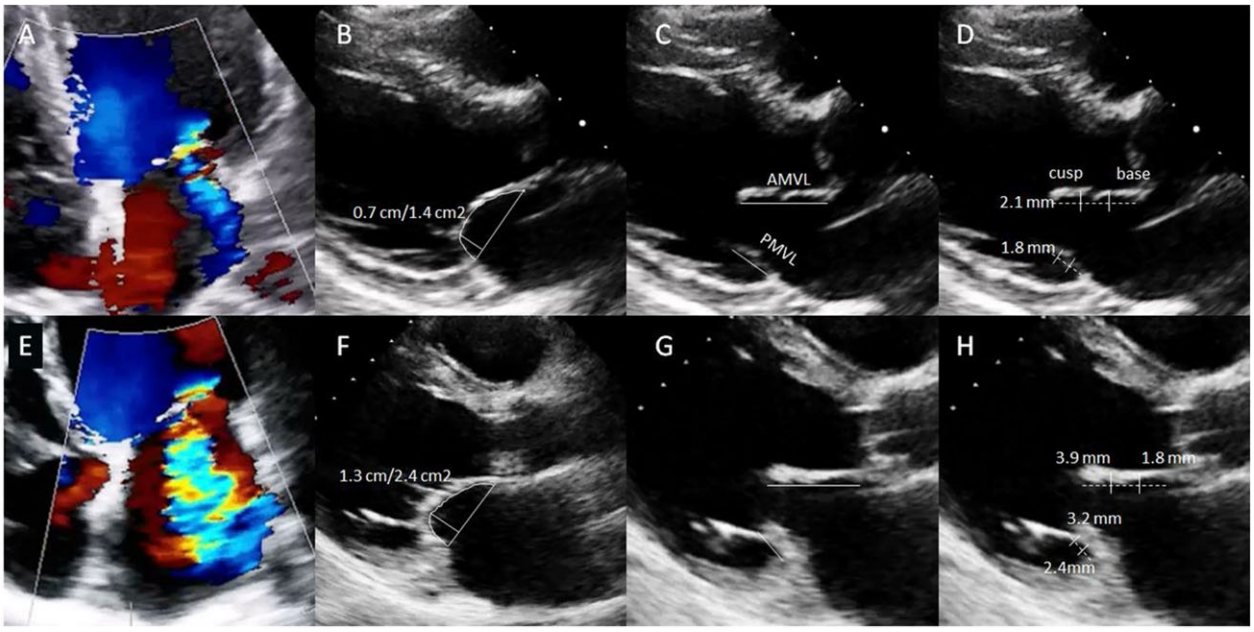


Figure 1 Echocardiographic mitral valve measurements in mild and severe. IMR Echocardiographic views of the MV from two patients with mild MR (top panel) and severe MR (bottom panel). Panels (*A* and *E*) shown in four-chamber view the jet of MR mild and severe, respectively. Panels (*B* and *F*) show measurement of coaptation depth and tenting area greater in (*F*). Panels (*C* and *G*) show the length of the AMVL and PMVL. In panel (*G*) note that the chord was excluded from the measurement of the PMVL length, which is short. Panels (*D* and *H*) show how leaflet thickness was measured. Leaflet length was divided into three equidistant segments. Note that the leaflets are markedly thickened at the cusp compared with the base (*H*) in severe MR compared with (*D*).

TTE imaging protocol was followed as outlined in the current guidelines for a comprehensive 2D TTE.[7]

MV length, tenting area, and coaptation depth

In the parasternal long-axis (PLAX) view, the anterior and posterior MV leaflets' length (AMVL and PMVL) were evaluated at end-diastole (ED), taking care not to include the chords in the measurement. In the same PLAX view, the anteroposterior diameter (AP) of the mitral annulus was measured in ED and in the proto-systolic phase, the ratio of these two diameters was expressed as a shortening fraction to describe annular dynamicity. Subsequently, the image was advanced to end-systole (ES) to measure the tenting area (defined as the area enclosed between the atrial surface of the valve leaflets and a connecting line point on the anterior and posterior mitral annulus) and the coaptation depth or tenting height (defined as the orthogonal distance between the annular plane and point of leaflet coaptation).

Echocardiographic MV thickness

MV thickness was measured in mid diastole in PLAX view with leaflets perpendicular to the echocardiographic beam in a frame without valve movement. Each leaflet was divided into three equidistant regions: base, middle, and cusp (tip). Three measurements were made at the base and three at the cusp to ensure that the thickest portion of each region was included in the measurements. The average of the three measurements in each region was used to indicate the thickness of the region (*Figure 1*). In a subset of 30 patients, length and thickness were measured twice by two independent physicians and one observer to assess inter-observer and intra-observer reproducibility.

Evaluation of mitral regurgitation severity

Single regurgitant jets were evaluated with quantitative methods such as the flow convergence method (PISA). If two or more jets were found, the severity assessment was evaluated using the Doppler continuity method, in accordance with the recommendations for the evaluation of valvular regurgitation. Both the PISA and continuity method considered an effective regurgitant orifice area (EROA) $\geq$0.40 cm^2 and regurgitant volume (RVol) $\geq$60 mL as severe; EROA of 0.20–0.39 cm^2 and RVol of 30–59 mL was considered moderate, and EROA < 20 cm^2 and <30 mL of RVol, was considered as mild.[8]

Histopathological analysis of leaflets

Following the current guidelines,[9] 48 patients underwent mitral valve replacement (MVR). The leaflets were carefully removed and placed in 10% buffered formalin, embedded in paraffin, cut into 5 µm sections, and mounted on glass slides, followed by stained with Masson's trichrome. The four-valve layers and their cellular composition were analysed. The histological composition was evaluated by microscopy in 10 thicker areas along the AMVL and PMVL, and similarly, the thickness at the bases and tips was measured. A single expert pathologist evaluated the thickness of the leaflets. AMVL and PMVL values were averaged to obtain a single value.

Table 1 Demographic and echocardiographic findings in the study population (echo-group)

	Group 1 ≤mild MR *n* = 148	Group 2 ≥moderate MR *n* = 102	*P*-value
Male (%)	130 (88)	83 (81)	0.15
Age (years)	60 ± 10	65 ± 10	<0.01
Weight (years)	77 (68–85)	73 (62–82)	<0.001
Height (m)	1.6 (1.6–1.7)	1.6 (1.6–1.7)	0.19
Body surface area (kg/m^2)	1.85 ± 0.19	1.80 ± 0.18	0.03
Hypertension (%)	68 (46)	64 (62)	<0.01
Dyslipidaemia (%)	50 (34)	49 (48)	0.02
T2D (%)	60 (40)	45 (44)	0.57
Hypothyroidism (%)	7 (5)	4 (4)	0.51
Smoking (%)	79 (53)	62 (61)	0.17
Echocardiographic findings			
EDD (cm)	4.5 ± 0.5	4.8 ± 0.5	<0.01
ESD (cm)	3.2 ± 0.6	3.8 ± 0.8	0.01
IVS (cm)	1.09 ± 0.2	1.09 ± 0.2	0.17
LVPW (cm)	1 (0.9–1.1)	1 (0.9–1.1)	0.61
LV mass (g/m^2)	91 (76–112)	105 (91–132)	<0.01
Wall motion score index	1.3 (1.1–1.5)	1.4 (1.2–1.7)	<0.01
Sphericity index	1.89 ± 0.2	1.9 ± 0.2	0.3
EDV (mL)	104 (87–125)	109 (91–136)	0.22
ESV (mL)	49 (38–60)	56 (41–84)	<0.01
EDVi (mL/m^2)	58 (47–69)	61 (49–67)	0.05
ESVi (mL/m^2)	27 (21–34)	32 (24–44)	<0.01
LVEF (%)	53 (49–58)	48 (40–55)	<0.01
LAVI (mL/m^2)	30 (26–37)	41 (33–53)	0.01
Basal RV diameter (mm)	36 (33–40)	38 (34–42)	0.01
Mean RV diameter (mm)	24 (21–28)	26 (21–32)	0.02
RV length (mm)	75 ± 8	73 ± 11	0.10
PASP (mmHg)	29 (25–34)	38 (30–53)	<0.01
Echocardiographic mitral valve findings			
EROA (cm^2)	0.12 (0.1–0.16)	0.35 (0.24–0.5)	
Regurgitant volume (mL)	15 (12–22)	57 (37–79)	
Mitral annulus diastole (mm)	27 (24–30)	30 (27–34)	<0.01
Mitral annulus systole (mm)	23 (20–25)	26 (22–30)	<0.01
Shortening fraction (%)	28 (21–35)	24 (19–33)	0.12
Tenting area (cm^2)	1.21 (0.9, 1.5)	1.65 (1.3, 2.1)	<0.01
Coaptation depth (mm)	0.76 (0.6–0.93)	0.94 (0.8–1.1)	<0.01
AMVL thickness (cm)	0.28 (0.23–0.33)	0.4 (0.31–0.48)	<0.001
PMVL thickness (cm)	0.28 (0.22–0.30)	0.38 (0.31–0.43)	<0.001
AMVL length (mm)	25 (23–28)	24 (22–28)	0.36
PMVL length (mm)	17 (15–19)	17 (14–18)	0.30

AMVL and PMVL, anterior and posterior mitral valve leaflets; EDD, end-diastolic diameter; EDV, end-diastolic volume; EDVi, end-diastolic volume indexed; EROA, effective regurgitant orifice area; ESD, end-systolic diameter; ESV, end-systolic volume; ESVi, end-systolic volume indexed; IVS, interventricular septum; LAVI, left atrial volume indexed; LV mass, left ventricular mass; LVEF, left ventricular ejection fraction; LVPW, left ventricular posterior wall; PASP, pulmonary arterial systolic pressure; RV, right ventricle; T2D, type 2 diabetes.

Data analysis

Patients were divided according to IMR severity into two groups for analysis. Group 1 included absent and mild IMR, while Group 2 consisted of those with moderate to severe IMR. For the second analysis, patients who underwent MVR were divided based on the median thickness of the AMVL and PMVL measured by histopathology of the explanted valves.

Statistical analysis

Categorical variables were expressed as proportions, and comparisons were made using non-parametric tests. The continuous variables were evaluated with the Kolmogorov–Smirnov test to determine the normality of distribution. Normally distributed data were expressed as mean and standard deviation, and Student's *t*-test was used to compare the variables. If the distribution was not normal, the Mann–Whitney *U* test was

Table 2 Demographic, clinical, echocardiographic, and histological data of patients who underwent MVR

	Leaflet thickness <0.42 mm ($n = 21$)	Leaflet thickness ≥0.42 mm ($n = 27$)	*P*
Male (%)	15 (71)	22 (81)	0.49
Age (years)	66 ± 8	67 ± 8	0.8
Hypertension (%)	17 (80)	18 (67)	0.26
Dyslipidaemia (%)	14 (66)	17 (63)	0.79
T2D (%)	9 (42)	13 (47)	0.71
Hypothyroidism (%)	2 (9)	2 (7)	0.59
Smoking (%)	15 (71)	18 (67)	0.66
Echocardiographic findings			
EDD (cm)	5.17 ± 0.65	5.41 ± 0.53	0.16
ESD (cm)	3.85 ± 0.86	4.32 ± 0.79	0.05
IVS (cm)	1.06 ± 0.16	1.01 ± 0.15	0.29
LVPW (cm)	0.96 ± 0.20	0.94 ± 0.16	0.73
LV mass (g/m^2)	109 ± 22	122 ± 33	0.12
WMSI	1.4 (1.2–1.5)	1.6 (1.4–1.8)	0.001
LAVI (mL/m^2)	46 (36–59)	51 (45–60)	0.38
Basal RV diameter (mm)	36 ± 5.7	41 ± 5.2	0.01
Mid RV diameter (mm)	28.2 ± 5.8	29.8 ± 6.6	0.38
RV Length (mm)	73.6 ± 6.7	75 ± 6.7	0.47
PASP (mmHg)	46.7 ± 17.2	55.1 ± 19.1	0.12
Mitral annulus diastole (mm)	32.6 ± 5.7	33.9 ± 6.1	0.44
Mitral annulus systole (mm)	29.6 ± 6.4	29.1 ± 5.8	0.77
Shortening fraction (%)	0.09 ± 0.07	0.14 ± 0.07	0.06
Tenting area (cm^2)	1.8 (1.4–2.15)	2.1 (1.6–2.59)	0.16
Coaptation depth (mm)	1 (0.9–1.1)	1.1 (0.9–1.3)	0.33
Sphericity index	1.96 ± 0.18	1.79 ± 0.13	<0.01
EDVi (mL/m^2)	51 (46–66)	77 (65–93)	<0.01
ESVi (mL/m^2)	30 (26–38)	42 (33–63)	0.01
LVEF (%)	48 (41–54)	42 (35–52)	0.05
EROA (cm^2)	0.46 (0.38–0.72)	0.46 (0.37–0.72)	0.95
Regurgitant volume (mL)	73 (62–96)	80 (59–123)	0.74
AMVL thickness (cm)	0.32 ± 0.06	0.47 ± 0.04	<0.0001
PMVL thickness (cm)	0.31 (0.26–0.35)	0.42 (0.40–0.46)	<0.01
AMVL length (mm)	23 ± 2.7	25 ± 3.9	0.08
PMVL length (mm)	14 (13.5–16.5)	16 (14–17)	0.23
Histological analysis			
Thickness, base (mm)	1 (0.7–1.4)	1.3 (1–2)	0.02
Thickness cusps (mm)	2.2 (2–2.95)	3.2 (3–3.6)	<0.001
Myxoid tissue (%)	66 ± 12	53 ± 21	0.01
Fibrosis (%)	33 ± 12	40 ± 17	0.14

AMVL and PMVL, anterior and posterior mitral valve leaflets; EDD, end-diastolic diameter; EDV, end-diastolic volume; EDVi, end-diastolic volume indexed; EROA, effective regurgitant orifice area; ESD, end-systolic diameter; ESV, end-systolic volume; ESVi, end-systolic volume indexed; IVS, interventricular septum; LAVI, left atrial volume Indexed; LV mass, left ventricular mass; LVEF, left ventricular ejection fraction; LVPW, left ventricular posterior wall; PASP, pulmonary arterial systolic pressure; RV, right ventricle; T2D, type 2 diabetes.

used. Statistical significance was considered when the *P* was <0.05. Multivariable logistic regression analysis was used to identify parameters associated with the thickening of the leaflets.

Results

A total of 977 patients diagnosed with IMI were identified. Of these, 91 had acute IMI without PCI, 313 had significant lesions in other coronary arteries, 89 had a different infarct area, 128 had other significant valvular lesions, 74 patients had other exclusion pathologies or did not have adequate images for evaluation. Two hundred eighty-two patients met the inclusion criteria, but 32 patients with a sphericity index ≤1.5 were eliminated, leaving a total of 250 included in the study. These patients were further included in the echocardiography group. During the study period, 48 of them (19.2%) underwent surgical MVR for significant IMR. MV leaflets were carefully removed,

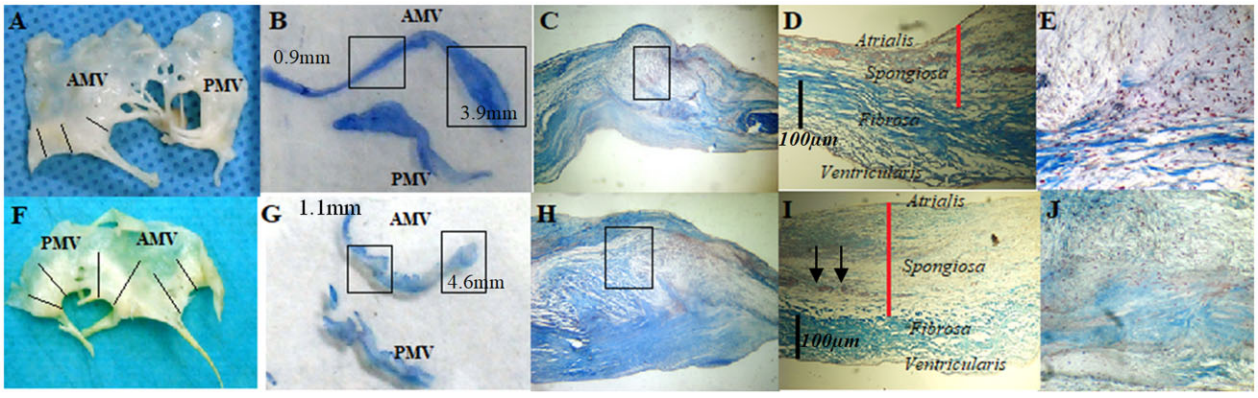


Figure 2 Explanted MV structure and histology in two patients with ≥moderate IMR, top panel leaflet thickness <0.42 cm and bottom with ≥0.42 cm. (*A*) The cusp of both leaflets was thickened and opaque (black lines) whereas the bases of the leaflets are thinner and transparent. (*B*) Longitudinal section of the leaflets (from base to tip) showing increased thickness of both cusps (AMV cusp was 3.9 mm thick, and the base was 0.9 mm thick); the rest of the valve was thin. (*C*) Cusp of AMV (big rectangle of image *B*), with Masson trichrome stain, shows thickening due to proliferation of myxoid tissue (in white) Masson 2.5×. (*D*) Middle part of the anterior leaflet (small rectangle in image *B*), shows a slight increase in the spongy layer (red line), vascular neoformation stands out in the atrialis layer (arrow). (*E*) Representation of the main tissue content of the cusp of the anterior leaflet, highlights the abundant myxoid tissue (white), rectangle in (*C*). The red mottling represents myofibroblasts and the blue lines are collagen fibres, which show disorder and are lost and replaced by myxoid tissue indicated by the increase in the spongiosa layer. The whole image represents the rectangle of the image *C* magnified 60×. (*F*) Explanted MV showing marked thickening of cusps of both leaflets (black lines). Note that the thickening extends almost to the base of the PMVL, and to the mid-portion of the AMVL. (*G*) Longitudinal section of the leaflets showing both leaflets are thickened from cusps to the base (AMVL cusp is 4.6 mm thick, and the base is 1.1 mm thick). (*H*) Cusp analysis (large rectangle in image *G*). Histology of the cusp of the AMVL with Masson trichrome stain shows severe thickening due to proliferation of myxoid tissue (in white), but also there was deposition of fibrous tissue in the spongiosa and in the atrialis layer, watch the blue lines in the spongiosa (blue arrow). The considerable increase in the thickness of the atrialis layer was due to fibrous tissue in blue colour (red arrow). Masson 2.5×. (*I*) Masson staining from the middle portion of the AMLV, shows a marked expansion of the spongiosa layer with myxoid tissue (red line), there is neovascularization in spongiosa (black arrows), and the blue areas in the spongiosa and atrialis layer represent areas of fibrosis that contribute to the increased thickness of the leaflet *J*. Representation of the tissue content of the cusp of the AMVL, highlights the abundant fibrous tissue stands out (blue), laid over the myxoid tissue of which some areas are still visible (white), rectangle in (*H*). The red mottling represents myofibroblasts (60×).

Table 3 Echocardiographic leaflet remodelling according to the degree of MR

	Without MR ($n = 92$)	Mild MR ($n = 56$)	≥ Moderate MR ($n = 102$)	*P*
PMVL tdickness (cm)	0.25 (0.21–0.29)	0.30 (0.25–0.32)	0.38 (0.31–0.43)	<0.0001[a,b]
AMVL lengtd (mm)	25.3 (23–28)	24.9 (22.2–29.4)	24.3 (22.9–28)	0.65
PMVL lengtd (mm)	17 (15–19)	16.8 (14.8–19.1)	16.8 (14.1–18.6)	0.50
AMVL thickness (cm)	0.26 (0.23–0.30)	0.31 (0.25–0.36)	0.41 (0.32–0.48)	<0.0001[a]

[a]No MR vs. ≥moderate MR.
[b]Mild vs. moderate MR.

histologically analysed, and included in a new group (MV histology-group).

Echocardiography group

The patients were grouped by IMR severity into Groups 1 and 2, as shown in *Table 1*. The patients in Group 2 were older with a lower weight and body mass index, and a greater portion had hypertension and dyslipidaemia ($P < 0.05$ for all). In the echocardiographic evaluation, Group 2 had larger end-diastolic, and end-systolic diameter (EDD, ESD), indexed left atrial volume (LAVI), and right ventricle (RV) basal and mid diameters. End systolic volume (ESV), indexed end-systolic volume (ESVi), LV mass, wall motion score index (WMSI), and pulmonary arterial systolic pressure (PASP) were more extensive as well ($P < 0.05$ for all). Left ventricular ejection fraction (LVEF) was minor in Group 2 $P < 0.01$. The diastolic and systolic MV annulus, the tenting area, and coaptation depth were larger in Group 2, $P < 0.01$ for all. Finally, the leaflet thickness was greater in Group 2, $P < 0.001$ for both (*Figure 1*). The comparison between operated and non-operated patients is shown in Supplementary data online, *Table S1*.

Table 4 Logistic regression to predict ≥ moderate mitral regurgitation (echo-group)

	Wald index	OR (95% CI)	*P*
Hypertension	0.74	1.39 (0.65–2.97)	0.38
Dyslipidaemia	2.43	1.81 (0.85–3.84)	0.11
LV mass (g/m^2)	2.03	1.01 (0.99–1.02)	0.15
WMSI	0.44	1.53 (0.43–5.47)	0.50
RV basal size (mm)	0.48	0.98 (0.92–1.03)	0.48
LVEF (%)	2.87	0.95 (0.89–1.00)	0.09
Anterior valve thickness ≥3.5 mm	5.19	2.81 (1.15–6.84)	0.02
Posterior valve thickness ≥3.5 mm	21.2	10.6 (3.89–29)	<0.001
Age (years)	4.12	1.03 (1.001–1.07)	0.04
TSVi (mL/m2)	2.22	0.97 (0.93–1.009)	0.13
Tenting area (cm^2)	3.66	2.77 (0.97–7.88)	0.05
Coaptation depth (mm)	0.00	0.90 (0.09–8.72)	0.93

Table 5 Logistic regression to predict the thickness of mitral valve

	Wald index	OR (95% CI)	*P*
SPAP(mmHg)	4.81	1.02 (1.003–1.04)	0.02
Coaptation deptd	6.82	4.33 (1.44–13.04)	0.009
WMSI	3.30	2.57 (0.93–7.11)	0.06
Sphericity index	1.24	0.46 (0.12–1.78)	0.26
LVEF (%)	2.48	0.97 (0.93–1.008)	0.11
Dyslipidaemia	3.36	1.7 (1.01–3)	0.049

MV histology-group

Forty-eight patients underwent MVR, 27 ± 19 months after index MI (8–37 months). The Indication for surgery was symptomatic moderate to severe mitral regurgitation (MR). The median cusp thickness of both leaflets was 0.42 cm (0.26–0.58 cm). Patients were divided into two groups based on the median thickness: those with cusp thickness <0.42 cm in Group 1 and ≥0.42 cm in Group 2, as displayed in *Table 2*. Group 2 showed significantly worse WMSI, increased basal diameter of the RV, decreased sphericity index, increased EDVi, and ESVi. AMVL and PMVL thickness were greater in Group 2 by echocardiography. Correlations between echocardiographic and histological measurements are shown in Supplementary data online, Graphic S1.

Histological analysis of the leaflets

The thickness of the base was 1 vs. 1.3 mm in Group 1 versus group 2, respectively, $P < 0.02$. While the cusp thickness was 2.2 vs. 3.2 mm, $P < 0.001$ (*Table 2* and *Figure 2*). There was extensive myxoid tissue in both groups, with a higher predominance in Group 1 (66% vs. 53%, $P < 0.01$). However, leaflets with a thickness ≥0.42 mm were characterized by having fibrous tissue deposition in the spongiosa and atrialis layer in addition to the myxoid tissue, which is estimated to increase the thickness and stiffness of the leaflets. There was a constant involvement of the three leaflet segments in Group 2 when the cusps of the leaflets were ≥0.42 (*Figure 2*).

Predictors of severity of MR and MV thickness

Thicker leaflets were associated with a greater degree of MR, as shown in *Table 3*. The logistic regression to predict moderate to severe MR showed that the median leaflet thickness of 3.5 mm was independently associated with >moderate MR [odds ratio (OR) 2.81 (95% confidence interval (CI), 1.15–6.84), $P < 0.02$] and [OR 10.6 (95% CI, 3.89–29), $P < 0.001$], AMVL and PMVL, respectively. The age also was a factor associated [OR 1.03 (95% CI, 1.001–1.07), $P < 0.04$], *Table 4*. Lastly, logistic regression to predict thickness of the MV leaflets showed that dyslipidaemia [OR 1.7 (95% CI, 1.01–3), $P < 0.049$], PASP [OR 1.02 (95% CI, 1.003–1.04), $P < 0.02$], and coaptation depth [OR 4.33 (95% CI, 1.44–13.04), $P < 0.009$] were independently associated (*Table 5*).

Reproducibility of measurements

In the evaluation of the echocardiographic thickness of the MV, there were no significant inter- or intra-observer differences, as observed in Supplementary data online, *Table S2*.

Discussion

This study has highlighted that in IMR, echocardiographic leaflet thickening was associated with moderate to severe MR. Moreover, histopathological remodelling changes were observed, finding a combination of myxomatous and fibrous tissue deposition in thicker specimens and an increased spongiosa layer predominantly affecting the cusps. Dyslipidaemia, SPAP, and coaptation depth were independently associated with MV thickness. Henceforth, the present investigation seems to bridge echocardiographic and microscopic MV leaflet structural changes with IMR severity.

Ischaemic MR is a consequence of post-infarction MV remodelling. The mechanical stress imposed by papillary muscle tethering increases MV leaflet area and matrix thickness with cellular changes suggestive of endothelial–mesenchymal transformation (EMT).[2,10–12] In our study, the coaptation depth was independently predictive of increased leaflet thickness. However, tethering may not be a prerequisite for leaflet thickening and IMR development. Ischaemia in non-RCA territories and post inferior wall infarction LV remodelling have been shown to trigger leaflet remodelling. During the early post-infarction period, the increased thickness may be an adaptation to reduce stress on the leaflets. Collagen remodelling in this phase of valve thickening is characterized by increased procollagen, although the collagen concentration may also be reduced to maintain the pliability of the leaflets.[5] Conceivably, leaflets are thicker but pliable at this stage and can form an effective seal during systole preventing MR occurrence (*Figure 3*). This phenomenon might explain why some post inferior wall infarction patients did not develop IMR or only presented mild stages in this and previous studies. We also found that leaflet thickness was significantly less extent in the no to mild ischaemic MR group. Nonetheless, it was thicker than in normal individuals

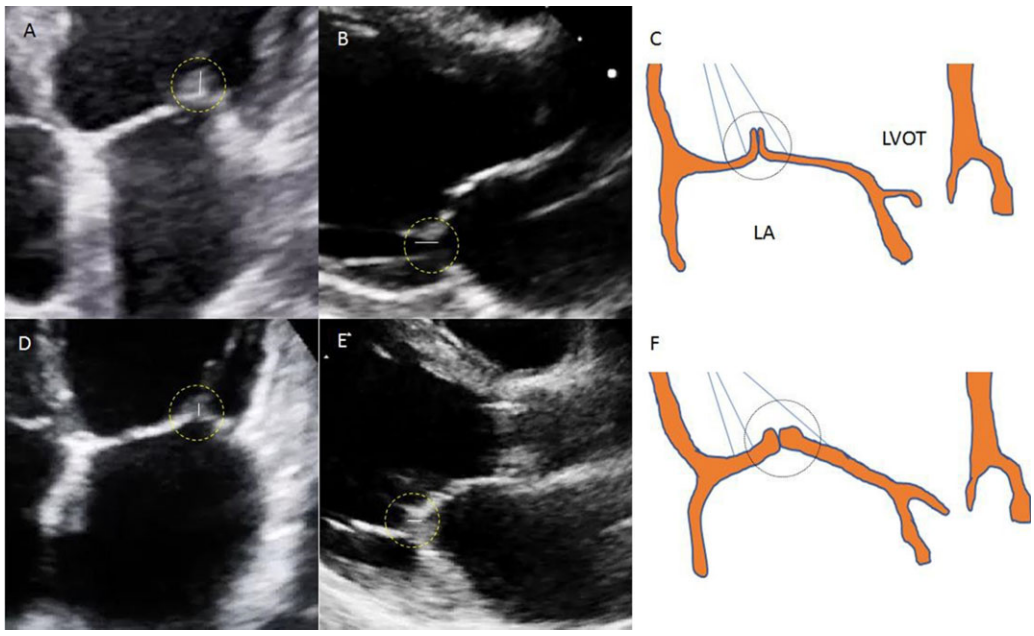


Figure 3 Mitral valve coaptation and sealing in mild (top panel) and ≥moderate (bottom) IMR. The mechanical effect of leaflet thickening on coaptation. In the upper panel, in mild IMR, although the leaflets are thickened, it retains its pliability and ability for the tips to bend to form an effective seal through a coaptation surface rather than a point. The length of the coaptation surface is indicated in (*A*) and (*B*) by the white lines. The illustration in (*C*) shows the bending of the leaflet tips to form the seal. In the lower panel, similar data are shown for severe IMR. Note that the length of coaptation surface is significantly short (white lines in *D* and *E*). The illustration in (*D*) shows that thickening of the leaflets especially at the cusps results in loss of the ability of the tips to bend to form a seal, instead they meet at a point.

described in other studies. Patients who underwent MVR had at least moderate ischaemic MR with thick AMVL and PMVL in all cases, especially in the cusps. Upon histopathological examination, cusps showed expansion of the spongiosa layer with an evident loss of collagen alignment and density and replacement by myxoid tissue. Although with MR progression, there is a generalized valvular thickening, it predominantly involves the cusps. This stiffening and fibrosis mechanism further limit valve closure, as previously described.[3]

When the cusp thickness exceeded 0.42 cm, we found that the markedly expanded spongiosa layer also exhibited dense infiltration with myofibroblasts, neovascularization, and fibrotic tissue, which was placed on the already increased myxoid tissue, making these leaflets more rigid. Conversely, only myxoid tissue was observed in cusps <0.42 cm. We hypothesized that the trend of containing more fibrosis over the myxoid tissue could indicate progression from myxoid to fibrotic degeneration. In this regard, animal studies have shown that collagen concentration increases with valve stiffening months after a MI.[5] Thus, the valves become stiffer, thicker, less mobile, and lose the ability to bend the tips at this stage. In this context, the interplay between leaflet thickness, stress, and pliability is complex. While the initial response is compensatory, continued tethering in the ischaemic environment may increase MV thickness, mainly in its cusps, which might be a mechanism that further contributes to the severity of ischaemic MR.

Moreover, this thickening was independently associated with dyslipidaemia as a cardiovascular risk factor and valvular coaptation depth as a mechanical process closely related to tethering. PASP was also associated as a predictor of thickening, possibly due to its association with the severity of MR. These cofactors are interesting points related to mechanical and functional variables that might have a close relationship with valvular stress, as the coaptation depth is related to the tethering on MV, which is a form of valvular stress.

Clinical implications

Ischaemic MR may begin as a ventricular disease. However, evidence of structural leaflet abnormalities has accumulated. Hence, this pathophysiological adaptative process might be relevant as it could be a therapeutic target (anti-inflammatory, anti-fibrotic, renin–angiotensin inhibition, among others) to manage the disease. Correspondingly, another intriguing question is the timing and appropriateness of valve repair or replacement in ischaemic MR. It is possible that this process could be associated with the benefit or not of valvular repair.

On the other hand, given that the patients with moderate to severe ischaemic MR group had relatively small LV and mildly reduced EF, they may represent a disproportionate functional mitral regurgitation (FMR) population. Thus, in a disproportionate FMR population, there may be patients with moderate MR and significant leaflet remodelling where medical therapy alone may not be sufficient to stop the progression of MR. Interestingly, a simple 2D echocardiogram can support the diagnosis of unbalanced mitral remodelling.

However, based on limited evidence on MV structural changes, these questions merit further prospective investigations.

Study limitations

This study was retrospective and cross-sectional. MVR was performed in these patients for IMR primarily due to our centre's limited expertise in repair techniques. Nonetheless, this is the most extensive histopathological report in explanted human MV from patients with IMR, and the findings are broadly along the line previously described in animals and humans. Whether the valve thickening may be due to shear leaflet stress due to MR is challenging to ascertain, as the leaflets remodelling occurs even in the absence of MR or post-infarction papillary muscle involvement.[3,10] Measurement of leaflet thickness by echocardiography can be challenging and requires good quality images. We focused on histology as it pertained to valve thickening. However, we did not study cellular changes or profibrotic signalling. We measured leaflet length but did not measure the leaflet area (a 3D measurement) or detailed annular or leaflet dynamics. Nevertheless, all of these have been extensively described in previous investigations. Finally, therapy was not evaluated, which could interfere with LV and leaflet remodelling. Some selection bias may occur in patients undergoing cardiac surgery inherent to this study's selection and analysis method.

Conclusions

In IMR, increased leaflets thickness, especially on the cusps, was associated with increased MR severity. In addition, this thickening was independently associated with depth of coaptation as a mechanical trigger and with dyslipidaemia and PASP as metabolic and haemodynamic triggers.

Histopathologically, the examined valves showed collagen breakdown and myxoid expansion of the spongiosa layer. However, as valvular thickening increased, a fibrosis deposition and more generalized involvement of the valves were observed in addition to the myxoid tissue. On the other hand, the thickness of the leaflets detected by echocardiography correlated with the histopathological remodelling changes. Therefore, this non-invasive diagnostic method could be potentially helpful in the evaluation of valvular remodelling, providing incremental value for timely valve intervention in IMR.

Supplementary data

Supplementary data are available at *European Heart Journal - Cardiovascular Imaging* online.

Conflict of interest: none declared.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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