Myocyte changes and their left atrial distribution in patients with chronic atrial fibrillation related to mitral valve disease

Domenico Corradi MDa,*, Sergio Callegari MDb, Stefano Benussi MD, PhDc, Roberta Maestri BsSc, PhDb, Paolo Pastori MDb, Simona Nascimbene MDe, Silvia Bosio MAd, Enrica Dorigo MDe, Chiara Grassani MAd, Raffaella Rusconi BsSce, Maria Vittoria Vettori BsSc, PhDe, Rossella Alinovi BsScf, Ettore Astorri MDg, Carlo Pappone MD, PhDh, Ottavio Alfieri MDe

Summary

It has been found that the pulmonary veins and adjacent left atrial posterior wall (LAPW) are deeply involved in both the initiation and maintenance of atrial fibrillation (AF), and the identification of these high-risk sites has aroused great interest in investigating their histopathologic substrate. We used light and conventional electron microscopy to evaluate the differential myocyte and interstitial changes in LAPW and left atrial appendage (LAA) samples from 28 patients with chronic AF undergoing mitral valve surgery and from 12 autopic controls. There were always more myocytes with loss of sarcomeres in the LAPW than in the LAA (19.9% vs. 8.2%; P < .0001), and the LAPW showed more marked immunohistochemical evidence of dedifferentiation, characterized by the reexpression of smooth muscle actin. In pathological left atria, myocyte diameter in the LAPW and LAA was comparable (19.0 ± 1.5 versus 18.5 ± 2.0 μm; not significant) but larger than in the controls (11.9 ± 0.8 and 12.1 ± 1.3 μm, respectively; P < .0001).
A terminal deoxynucleotidyltransferase assay did not reveal any myocyte apoptosis. The LAPW also showed more interstitial fibrosis than the LAA (7.49% ± 3.34% versus 2.80% ± 1.35%; P < .0001). Ultrastructural examination confirmed the presence of myocyte myocytolysis in the perinuclear area and showed changes in mitochondrial shape.

In conclusion, the LAPW in patients with chronic AF related to mitral valve disease seems to be a particular anatomical site in which major myocyte and interstitial changes are concentrated, whereas the LAA is more protected. This remodeling may increase the heterogeneity of LAPW electrical conduction, thus confirming this location as an elective target for the ablation treatment of AF.

**1. Introduction**

Atrial fibrillation (AF) is the most common sustained arrhythmia and affects approximately 2% of the adult population. This prevalence progressively increases with age up to 5.9% in subjects older than 65 years [1]. However, despite the introduction of new selective drugs, the pharmacological treatment of AF is still too often ineffective [2], and this has led to growing interest in identifying nonpharmacological management strategies aimed at restoring and maintaining sinus rhythm. At the same time, new mapping and imaging techniques have made it possible to identify the specific atrial sites involved in AF initiation: pulmonary veins (PVs) are characterized by greater spontaneous electrical activity [3,4], and the adjacent left atrial posterior wall (LAPW) plays a crucial role in the predisposition to develop and maintain sustained AF [5,6].

A recently introduced nonpharmacological approach to AF is radiofrequency catheter ablation, which is based on the assumption that a sufficient number of reentrant circuits to perpetuate AF cannot coexist without a large myocardial mass in which to operate and has the aim of creating long linear connecting scars that divide the myocardial atrial mass into electrically isolated areas that are too small to sustain the arrhythmia. The efficacy of this ablation procedure further confirms the role played by the left atrial (LA) myocardium around the PV in inducing and maintaining AF.

This increased understanding of AF has also generated great interest in its histopathologic substrate and electrophysiological consequences [7], thus giving rise to a new perspective in which it is no longer seen as a purely functional disease, but the result of various histological changes capable of sustaining it.

We have recently shown that there is greater interstitial fibrosis (IF) and lower capillary density in the LAPWs of patients with chronic AF (CAF) undergoing mitral valve surgery than in the corresponding left atrial appendage (LAA) or controls [8], which suggested nonhomogeneous atrial interstitial remodeling in subjects with AF who have mitral disease. Although a number of animal studies have described myocyte remodeling after artificially induced pure AF [9-15], this was the first to report the LA distribution of myocyte structural remodeling in patients with AF.

The aim of the present study was to evaluate myocyte and interstitial remodeling in 2 LA locations—the LAPW and LAA—in patients with CAF related to mitral disease (CAF-MD) and to investigate possible regional differences capable of explaining the greater propensity of the LAPW to induce and maintain AF.

**2. Methods**

**2.1. Patients with CAF-MD**

Left atrial posterior wall and LAA samples were taken from 28 patients with CAF during combined mitral surgery and LA radiofrequency ablation [16,17] performed at the Cardiothoracic Surgery Unit of San Raffaele Hospital, Milan, Italy. The intraoperative sampling was always carried out before performing the radiofrequency ablation lines. The study was conducted in accordance with a protocol approved by our Institutional Human Research Committee and respected the principles outlined in the Declaration of Helsinki. The samples (approximately 5 × 10 × 2 mm) were taken from the LAPW myocardium between the inferior and superior right PVs (adjacent to the atriotomy line) [16] and the body of the LAA (Fig. 1). The patient...
population consisted of 9 subjects with mitral stenosis (CAF-MS), 10 with mitral insufficiency (CAF-MI), and 9 with mitral stenosis and insufficiency (CAF-MSI), as assessed by means of clinical, echocardiographic [18], and Doppler criteria [19].

2.2. Controls

Left atrial samples excised from the same sites as those described previously were taken from 12 autopic hearts. All of the autopsies had been performed at the Department of Pathology and Laboratory Medicine of the University of Parma, Italy, within 24 hours of death and in accordance with the current Italian legislation. The exclusion criteria included medical records of any type of arrhythmia, valvular disease, atherosclerosis of the major coronary arteries with less than 30% lumen reduction, acute or healed myocardial infarction, heart hypertrophy (>400 g for women and >450 g for men), metastatic neoplasm, multiple myocardial foci of replacement fibrosis less than 2 mm in diameter, inflammatory cells in the myocardial interstitium, sarcoidosis, amyloidosis, chronic inflammatory respiratory diseases, and connective tissue disorders [8,20].

2.3. Tissue sampling for morphological and morphometric investigations

After excision, the specimens were fixed in 10% buffered formalin solution for 24 to 48 hours and embedded in paraffin blocks. The blocks were then sectioned at a thickness of 5 μm, stained with hematoxylin-eosin, Masson trichrome, and periodic acid–Schiff (PAS) combined with toluidine blue, and observed through a light microscope (Olympus BX51, Tokyo, Japan).

Additional 5-μm histological sections were used for immunohistochemical analyses using antibodies against smooth muscle actin (NeoMarkers, Fremont, Calif; dilution 1:200) and sarcomeric actin (DakoCytomation, Glostrup, Denmark; dilution 1:50).

Briefly, the sections were dewaxed in xylene, rehydrated through graded alcohol, and, after quenching endogenous peroxidase with a 3% H2O2 water solution, incubated with a protein block (Ready to Use DakoCytomation Biotin Blocking System, DakoCytomation California Inc, Carpinteria, Calif). They were then heated in citrate buffer (pH 6.0) at four 5-minute microwave cycles.

To test the primary antibodies, the slides were incubated for 30 minutes at room temperature, and the reactions were revealed using streptavidin-biotin-peroxidase complex (K0675, LSAB 2 System, DakoCytomation) followed by a solution of 3-3’-diaminobenzidine tetrahydrochloride. Finally, they were counterstained with Mayer hematoxylin, mounted, and coverslipped. The negative control procedure consisted of the omission of the primary antibody.

Myocyte diameter was morphometrically evaluated on hematoxylin-eosin–stained sections in all case. For each sample, a digital photocamera (Olympus DP11, Tokyo, Japan) at a magnification of ×400 was used to take up to 10 images to evaluate longitudinally oriented myocytes. The shorter axis was carefully measured in the region of the nucleus using Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, Md).

2.4. Myocytes and myocytolysis quantification

The percentage of myocytes showing a perinuclear myofibril loss of greater than 25% [14], with or without glycogen substitution, was quantified on PAS and toluidine blue–stained histological sections at a magnification of ×400 by counting the fraction of affected cells in 30 LAA and 30 LAPW microscopic fields in each heart. Myocytolysis of greater than 25% was determined by dividing the

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics and echocardiographic data</th>
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<tr>
<td>No. of patients</td>
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<td>Age (y)</td>
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<td>Sex (M/F)</td>
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<td>Body surface area (m^2)</td>
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<td>CAF duration (mo)</td>
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<td>LVEDD (mm)</td>
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<td>LVEDD (mm)</td>
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<td>LVEF (%)</td>
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<td>LA-SID (mm)</td>
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<td>LAA-NW (mm)</td>
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<tr>
<td>LAA length (mm)</td>
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<td>PAPs (mm Hg)</td>
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</table>

NOTE. Data are expressed as means ± SD, with ranges in brackets.

Abbreviations. M, male; F, female; LVEDD, left ventricle end-diastolic diameter; LVEDD, left ventricle end-systolic diameter; LVEF, left ventricle ejection fraction; SID, superior-inferior dimension; MLD, medial lateral dimension; NW, neck width; PAPs, pulmonary artery systolic pressure.
perinuclear area (characterized by myofibril loss) by the entire cytoplasmic area using micrometric ocular lens (Olympus WH10X-H/22) at a magnification of \( \times 1000 \).

### 2.5. Deoxyuridine triphosphate nuclear labeling

Deoxyuridine triphosphate nuclear labeling (which indicates DNA-strand breaks consistent with apoptosis) was evaluated by means of a terminal deoxynucleotidyltransferase assay using the streptavidin–horseradish peroxidase (HRP) DAKO LSAB 2 System kit (DakoCytomation), as previously described [21].

### 2.6. Myocardial fibrosis quantification

The percentages of the myocardium occupied by myocytes, normal interstitium (matrix, vessels, nerves, etc), IF, and perivascular fibrosis (PF) were evaluated at a magnification of \( \times 400 \) in Masson trichrome method–stained histological sections using a light microscope equipped with an ocular reticule containing 42 sampling points (no. 105844, Wild Heerbrugg Instruments, Inc, Heerbrugg, Switzerland). The fractions of ocular reticule points overlying myocytes, normal interstitium, IF, and PF were accurately counted [8,22] and then multiplied by 100. A total of 35 LAA and 35 LAPW microscopic fields of each left atrium were measured [8].

### 2.7. Ultrastructural examination

An ultrastructural examination was made of the LAPW and LAA samples (approximately \( 1 \times 1 \) mm) from 8 patients with CAF-MD and 5 controls, using previously described techniques [8]. Each of these myocardial

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**Fig. 2**  
A, LAPW in a patient with CAF-MD. Myocardial architecture is diffusely altered by enlarged myocytes and a marked intercellular collagen network (hematoxylin-eosin, original magnification \( \times 20 \); scale bar, 20 \( \mu \)m). B, C, and D, Photomicrographs of LAPW (B) and LAA (C) samples from a patient with CAF-MD. The LAPW has more myocytolytic myocytes with (arrow) or without (arrowhead) glycogen substitution than the LAA. There are no myocytolytic myocytes in a control LAPW (D) (PAS combined with toluidine blue, original magnification \( \times 10 \); scale bar, 100 \( \mu \)m). E and F, Details of a pathological LAPW showing 2 myocytolytic myocytes with (E) or without glycogen substitution (F) (PAS combined with toluidine blue, original magnification \( \times 40 \); scale bar, 30 \( \mu \)m).
fragments was cut from the fresh tissue sample intended for light microscopy analysis before the fixation process.

2.8. Statistical analysis

The data are expressed as means ± SD. The normal distribution of the morphometric values was verified using the Kolmogorov-Smirnov test, and between-group differences were assessed by means of 1-way analysis of variance and Student t test for paired and unpaired data. The morphometric measurements of the patients and controls were analyzed by means of linear regression using Pearson correlation coefficients. All of the tests were performed using SPSS version 12.0 software (SPSS Inc, Chicago, Ill). P values of less than .05 were considered statistically significant.

3. Results

3.1. Patients with CAF-MD

The 28 patients (7 men and 21 women) were divided into 3 groups on the basis of the associated valvulopathy: 9 with CAF-MS, 9 with CAF-MSI, and 10 with CAF-MI.

Table 1 shows their general data and main echocardiographic measurements. Eighteen had a related rheumatic mitral disease, and the remaining 10 had degenerative valvulopathy. The age and body surface area of the patients in the 3 groups and the controls were comparable. Before heart surgery, 3 patients were in New York Heart Association Heart Failure Classification class I, 15 in class II, and 10 in class III.

3.2. Controls

The control autopic LA samples were collected from 6 men and 6 women aged 40 to 79 years (average, 64 ± 12 years); their body surface area ranged from 1.56 to 1.85 m² (average, 1.68 ± 0.20 m²). Total heart weight averaged 383 ± 52 g (range, 240-440 g).

Macroscopic and histological examinations excluded all of the diseases considered as exclusion criteria.

The causes of the deaths of the controls were traumatic injury caused by car accidents (4 cases), cerebral hemorrhage (4 cases), retroperitoneal hemorrhage (3 cases), and acute pyogenic meningitis (1 case).

3.3. Myocytes and myocytolysis quantification

The myocardial architecture of the pathological atria was diffusely altered by the presence of enlarged myocytes and a marked intercellular collagen network (Figs. 2A and 3). Table 2 shows the percentages of myocytes with perinuclear myofibril loss with or without glycogen substitution (Fig. 2B and C). Although

<table>
<thead>
<tr>
<th></th>
<th>LAPW myocyte diameter</th>
<th>LAA myocyte diameter</th>
<th>LAPW myocytes with myocytolysis (%)</th>
<th>LAA myocytes with myocytolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients with CAF-MD</strong></td>
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<tr>
<td>CAF-MS</td>
<td>19.0 ± 1.7* (17.1-21.2)</td>
<td>19.0 ± 2.9* (16.9-26.4)</td>
<td>21.6 ± 7.6*† (11.1-32.9)</td>
<td>10.8 ± 3.8* (6.3-18.2)</td>
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<tr>
<td>CAF-MSI</td>
<td>18.6 ± 1.5* (16.3-20.3)</td>
<td>17.8 ± 1.4* (15.6-19.3)</td>
<td>18.0 ± 5.9*† (8.7-25.8)</td>
<td>7.5 ± 5.5* (0.0-15.7)</td>
</tr>
<tr>
<td>CAF-MI</td>
<td>19.0 ± 1.5* (16.3-21.1)</td>
<td>18.4 ± 1.1* (16.7-19.9)</td>
<td>19.0 ± 8.2*† (6.9-33.8)</td>
<td>6.7 ± 3.9* (1.3-12.1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>19.0 ± 1.5* (16.3-21.2)</td>
<td>18.5 ± 2.0* (15.6-26.4)</td>
<td>19.9 ± 7.7*† (6.9-33.8)</td>
<td>8.2 ± 5.0* (0.0-18.2)</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>11.9 ± 0.8 (10.6-13.3)</td>
<td>12.1 ± 1.3 (10.2-14.8)</td>
<td>0.8 ± 0.9 (0.0-2.8)</td>
<td>0.6 ± 0.7 (0.0-2.3)</td>
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NOTE: Statistical results. Data are expressed as means ± SD, with ranges in brackets.

* P < .001 compared with controls.
† P < .0001 compared with corresponding LAA sample.
to varying degrees and regardless of the underlying mitral defect, the LAPW fraction in each patient with CAF-MD always exceeded that of the LAA; the control cases all had a negligible fraction of myocytolytic myocytes (Fig. 2D). The myocytolytic and nonmyocytolytic cells were similar in size (Figs. 2E and 1F). The number of cells with glycogen accumulation was not related to the total number of myocytolytic cells in any of the cases; furthermore, no statistical correlations were found between the percentages of myocytolytic myocytes and age, CAF duration, New York Heart Association class, the percentages of IF and PF, or the echocardiographic measurements.

3.4. Myocyte diameter

Myocyte diameter was greater in the patients with CAF-MD than in the controls (Fig. 4), with no differences between the diameters of the LAPW and LAA myocytes (Table 2).

3.5. Immunohistochemistry

Antisarcomeric actin primary antibody was diffusely positive in the LAPW and LAA of the pathological (Fig. 5A and C) and control samples, whereas smooth muscle actin was partially expressed in the pathological but not in the control LAPW and LAA samples (Fig. 5B and D).
Quantitatively, the immunohistochemical positivity for smooth muscle actin followed the distribution of the myocytolytic cells.

### 3.6. Deoxyuridine triphosphate nuclear labeling

Deoxyuridine triphosphate nuclear labeling was only found in rare interstitial cells in both the pathological and control samples. The myocytes were always negative.

### 3.7. Myocardial fibrosis quantification

Expressed in terms of its percentage in the myocardium or its ratio to myocytes, there was more IF in the LAPW than the LAA samples from the patient groups than in the control samples, with no differences between the 3 patient groups. On the contrary, there was no difference in PF between the 3 patient groups and controls (Table 3).

### 3.8. Ultrastructural examination

Ultrastructural examination showed that the remodeled myocytes were characterized by widespread sarcomere depletion, mainly in the perinuclear zone (Fig. 6A). When present, the myofilaments were often thin and irregular with widened Z bands (Fig. 6B). The empty spaces in the myocytolytic areas were filled with sparse sarcomeres or glycogen (Fig. 6C). Some mitochondria had an elongated shape with inconstant longitudinal orientation of the cristae (Fig. 6C and D). These changes were qualitatively similar in both the LAPW and LAA samples. No clear signs of cytoplasmic edema, vacuolization, or lipid droplets were found in the pathological specimens.

Most myocytes from the control atria were characterized by normally distributed sarcomeres with uniform mitochondria (Fig. 6E and F).

### 4. Discussion

The results of this study indicate that the structural remodeling of the left atrium in patients with CAF-MD involves particularly strong changes in the LAPW. In qualitative terms, a variable but significant number of myocytes show clear signs of myocytolysis as demonstrated as

### Table 3  Myocardial components

<table>
<thead>
<tr>
<th></th>
<th>LAPW</th>
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<th>IF/myocyte ratio</th>
<th>PF/myocyte ratio</th>
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<tr>
<td></td>
<td></td>
<td>Myocytes (%)</td>
<td>IF (%)</td>
<td>PF (%)</td>
<td>Other myocardial structures (%)</td>
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<tr>
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<tr>
<td>CAF-MS</td>
<td>87.33 ± 4.31</td>
<td>8.43 ± 4.21†</td>
<td>0.21 ± 0.27</td>
<td>3.93 ± 1.85**</td>
<td>0.100 ± 0.054†§</td>
</tr>
<tr>
<td></td>
<td>(82.90-93.66)</td>
<td>(3.53-14.78)</td>
<td>(0.00-0.48)</td>
<td>(1.53-6.71)</td>
<td>(0.039-0.177)</td>
</tr>
<tr>
<td>CAF-MSI</td>
<td>87.76 ± 3.01†</td>
<td>7.15 ± 2.99†</td>
<td>0.37 ± 0.18</td>
<td>4.72 ± 2.30</td>
<td>0.082 ± 0.037†§</td>
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<td></td>
<td>(82.84-91.37)</td>
<td>(3.59-12.40)</td>
<td>(0.08-0.74)</td>
<td>(1.20-7.53)</td>
<td>(0.040-0.150)</td>
</tr>
<tr>
<td>CAF-MI</td>
<td>87.38 ± 2.55</td>
<td>6.87 ± 2.71†</td>
<td>0.43 ± 0.46</td>
<td>5.32 ± 1.23**</td>
<td>0.080 ± 0.033†§</td>
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<td></td>
<td>(83.97-91.65)</td>
<td>(2.33-10.62)</td>
<td>(0.08-1.60)</td>
<td>(3.55-7.20)</td>
<td>(0.026-0.125)</td>
</tr>
<tr>
<td>Total</td>
<td>CAF-MD 87.49 ± 3.23</td>
<td>7.49 ± 3.34†</td>
<td>0.34 ± 0.31</td>
<td>4.68 ± 1.85†§</td>
<td>0.087 ± 0.041†§</td>
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<td></td>
<td>(82.84-93.66)</td>
<td>(2.33-14.69)</td>
<td>(0.00-1.60)</td>
<td>(1.20-7.53)</td>
<td>(0.026-0.177)</td>
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<tr>
<td>Controls</td>
<td>93.33 ± 1.13</td>
<td>1.10 ± 0.69</td>
<td>0.70 ± 0.39</td>
<td>4.86 ± 1.35</td>
<td>0.012 ± 0.007</td>
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<td>(91.46-94.71)</td>
<td>(0.19-1.89)</td>
<td>(0.24-1.44)</td>
<td>(3.17-7.07)</td>
<td>(0.002-0.020)</td>
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<tr>
<td>CAF-MS</td>
<td>94.49 ± 1.27</td>
<td>2.73 ± 1.49</td>
<td>0.30 ± 0.72</td>
<td>2.33 ± 1.13†</td>
<td>0.031 ± 0.020*</td>
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<td>(92.04-95.71)</td>
<td>(0.64-6.06)</td>
<td>(0.00-2.20)</td>
<td>(0.07-3.66)</td>
<td>(0.007-0.071)</td>
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<td>CAF-MSI</td>
<td>93.53 ± 2.00</td>
<td>2.48 ± 1.03♀</td>
<td>0.45 ± 0.53</td>
<td>3.54 ± 1.63</td>
<td>0.027 ± 0.011♀</td>
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<td>(89.72-96.58)</td>
<td>(1.11-3.88)</td>
<td>(0.00-1.48)</td>
<td>(2.12-7.34)</td>
<td>(0.012-0.042)</td>
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<tr>
<td>CAF-MI</td>
<td>92.88 ± 2.48</td>
<td>3.01 ± 1.22♀</td>
<td>0.35 ± 0.36</td>
<td>3.76 ± 1.37</td>
<td>0.033 ± 0.015♀</td>
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<tr>
<td></td>
<td>(86.41-95.08)</td>
<td>(2.15-6.18)</td>
<td>(0.00-1.22)</td>
<td>(1.92-6.92)</td>
<td>(0.023-0.072)</td>
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<td>Total</td>
<td>CAF-MD 93.60 ± 2.04</td>
<td>2.80 ± 1.35♀</td>
<td>0.37 ± 0.53</td>
<td>3.23 ± 1.48♀</td>
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<tr>
<td></td>
<td>(86.41-96.58)</td>
<td>(0.64-6.53)</td>
<td>(0.00-2.20)</td>
<td>(0.07-3.74)</td>
<td>(0.007-0.072)</td>
</tr>
<tr>
<td>Controls</td>
<td>93.65 ± 0.96</td>
<td>0.54 ± 0.50</td>
<td>0.59 ± 0.83</td>
<td>5.22 ± 1.39</td>
<td>0.006 ± 0.005</td>
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<tr>
<td></td>
<td>(92.18-94.85)</td>
<td>(0.00-1.33)</td>
<td>(0.07-2.03)</td>
<td>(3.09-7.38)</td>
<td>(0.000-0.014)</td>
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</table>

NOTE. Statistical results. Data are expressed as means ± SD, with ranges in brackets.

* P < .005 compared with controls.

** P < .0001 compared with corresponding LAA sample.

† P < .001 compared with controls.

‡ P < .01 compared with controls.

§ P < .05 compared with controls.

‖ P < .005 compared with corresponding LAA sample.

# P < .001 compared with corresponding LAA sample.

‡‡ P < .01 compared with controls.
by myofibril depletion in the perinuclear area, an empty space that PAS stain positivity showed to be sometimes filled with an accumulation of glycogen. Although not uniform, this loss of sarcomeres was invariably detected in each pathological sample, but the LAPW of each patient, regardless of the kind of mitral defect, always had a larger percentage of myocytolytic myocytes than the LAA (19.9% ± 7.7% versus 8.2% ± 5.0%). In addition to myocytolytic changes, we also found aspects of dedifferentiation, such as the reexpression of smooth muscle actin (typical of fetal myocytes) and an abnormal mitochondrial ultrastructure. Furthermore, the diameters of the myocytolytic and nonmyocytolytic elements of CAF atria were always larger than those observed in the controls, although unlike the other changes, the increased myocyte diameter was similar in both the LAPW and LAA samples from the patients.

In line with previously published data [8], the LAPW was also characterized by more IF than the LAA, whether this was assessed in terms of its percentage in the myocardium (7.49% ± 3.34% versus 2.80% ± 1.35%) or its ratio to myocytes (0.087 ± 0.041 versus 0.030 ± 0.015). Conversely, there were no differences in PF in the pathological or control cases.

The causes and functional meaning of the individual myocardial changes in the patients with CAF-MD are still unclear, as is their regional distribution. The morphological picture of LA samples from such patients usually represents the end point of various histological changes, each of which can be attributed to the valvulopathy, AF, or both.

Fig. 6  A, B, C, and D, Ultrastructural appearance of the LAPW in a patient with CAF-MD. A, The myocytolytic process mainly involves the myocyte perinuclear area, which is characterized by fragments of sarcomeres (arrow), granules of glycogen, and mitochondria (arrowhead). B, A fragmented myofilament with widened Z bands (arrow) is located in the myocytolytic space. C, Mitochondria are often characterized by an elongated (arrow) and occasionally “forked” shape (arrowhead). D, The cristae in these mitochondria often have a longitudinal orientation. E and F, Ultrastructural appearance of the LAPW in a control autopic heart. Myocyte architecture is preserved, with longitudinally oriented myofilaments and the absence of any sign of myocytolysis. There are some autolytic changes mainly involving mitochondria (original magnifications, ×2200 [A]; ×8900 [B]; ×14000 [C]; ×36000 [D]; ×3500 [E]; and ×7100 [F]).
It has been described in an experimental dog model that hemodynamic overload secondary to mitral disease causes progressive LA enlargement that leads to the deposition of connective fibers in a myocardium with hypertrophied myocytes, and these atria were consequently highly susceptible to the initiation and perpetuation of atrial arrhythmias, such as AF [23].

Recent studies have investigated the effects of induced pure AF, which is characterized by a rapid atrial rate and a lack of atrial contractile activity [11,13,24-26]. This status induces structural changes such as myocyte dedifferentiation, and IF.

In this context, a reduced number of working sarcomeres in a variable but significant number of atrial myocytes could make the atrial dysfunction progressively worse and induce a subsequent increase in passive atrial stretching, thus creating a sort of vicious feedback loop that is itself capable of perpetuating AF [25,27-29].

The meaning of the dedifferentiation process is not yet clear, but it is interesting to note that the phenotype of dedifferentiated myocytes in CAF shares a number of characteristics with a hibernating myocardium (eg, myocytolysis, myocyte reexpression of fetal proteins, mitochondrial changes), which is an adaptive response to reduced coronary blood supply [9,30]. As the LA interstitium in patients with CAF-MD is characterized by a reduced capillary density that leads to a greater oxygen diffusion distance in the myocardium [8], it can be hypothesized that, in cases of increased oxygen need, a potentially reduced oxygen supply may cooperate in inducing the dedifferentiated phenotype. However, unlike in the case of late-stage hibernating myocardium [31], we did not find any sign of deoxyuridine triphosphate labeling in myocyte nuclei, mitochondrial swelling, or lysosomal degradation, thus suggesting a partial adaptation to new electrical and working conditions at this stage of disease. These findings are in line with those observed in previous experimental models [32,33]; however, clinical studies of patients with CAF have described apoptotic myocytolytic elements, thus interpreting this picture as degenerative [34].

A unifying hypothesis is that the myocardial changes observed in AF may start as a reactive attempt to adapt the LA myocardium to a new state of loading and rhythm, but in the presence of severe clinical conditions, this remodeling induces irreversibly degenerative changes [35].

Although IF acts as a reparative mechanism after myocyte loss [36], some authors also consider interstitial collagen deposition a reactive phenomenon involving both angiotensin II–dependent and independent pathways [37]. At the same time, in AF, increased myocyte diameter may be the result of a process aimed at compensating for cell loss, as well as a hypertrophying response induced by angiotensin II through the activation of the angiotensin I receptors that are up-regulated in the LA of patients with AF [38].

In addition to being induced by both AF and MD, the changes characterizing anatomical remodeling in CAF-MD atria induce nonuniform intracellular and extracellular cardiac anisotropy and may themselves increase the heterogeneity of conduction and thus favor the maintenance of the arrhythmic disturbance [39]. It is in fact known that structural remodeling in the form of interstitial collagen deposition or a reduction in sarcomere content can lead to myocardial areas characterized by abnormally slow conduction, blocks, and reentries capable of stabilizing AF [40,41].

With regard to the regional distribution of the structural remodeling found in the present study, according to Laplace law, we can hypothesize that the LAPW undergoes greater wall stress than the LAA. This is because, although the blood pressures in the left atrium and LAA are similar, the LAPW is thinner than the LAA, and the volume of the LA chamber is larger than that of the LAA [42]. In addition, the different reactions to wall stress may be partially caused by the different embryological origins of the 2 regions: the LAA develops from the embryonic left atrium, whereas the LAPW originates from the outgrowth of the PVs [8,43].

Another crucial point is that the myocyte and interstitial changes capable of favoring and sustaining the arrhythmia are only partially reversible after the restoration of sinus rhythm. For example, Ausma et al [40] observed only a partial recovery from structural remodeling in the goat model: 4 months after sinus rhythm recovery, myocyte diameters were completely restored, whereas the changes in the expression of structural proteins were only partially restored, and myocytes with a mild loss of myofibrils could still be detected. Moreover, interstitial collagen tissue deposition did not change during the 4 months after AF. The question remains as to whether longer periods after CAF resolution may be necessary to ensure complete recovery from myocardial remodeling.

In conclusion, the results of this study demonstrate that the myocardium of the LA is modified by significant remodeling in patients with CAF-MD undergoing valvular surgery. Major histological changes seem to be concentrated in the LAPW, whereas the LAA is more protected from the deleterious effects of both the arrhythmia and the mitral disease. The reduction in myocyte sarcomeric content, together with noticeable interstitial changes such as fibrosis and impaired capillary density, may themselves sustain AF by increasing the heterogeneity of LAPW electrical conduction, thus confirming this anatomical location as a specific target for the ablation treatment of AF.

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