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## Regional left atrial interstitial remodeling in patients with chronic atrial fibrillation undergoing mitral-valve surgery

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**Abstract** Ablation of the left atrial free wall around the pulmonary vein ostia (LAFW) may be effective in the treatment of chronic atrial fibrillation associated with mitral disease (CAF-MVD). Using light and conventional electron microscopy analyses, we wanted to evaluate, in CAF-MVD, the interstitial remodeling in the LAFW as well as in a more remote region, such as the left atrial appendage (LAA). LAFW and LAA samples were obtained from 33 CAF-MVD patients during combined mitral surgery and radiofrequency ablation and from 16 autotopic controls. Interstitial fibrosis (IF) and perivascular fibrosis (PF), capillary densities and the maximal oxygen diffusion distance were morphometrically determined. In CAF-MVD patients, the LAFW, compared with the LAA, showed a higher percentage of IF ( $7.16\pm 3.23\%$  versus  $2.51\pm 1.40\%$ , respectively), a lower myocardial capillary

density per  $\text{mm}^2$  ( $830\pm 106$  versus  $989\pm 173$ ) and an increased oxygen maximal diffusion distance ( $19.70\pm 1.27\ \mu\text{m}$  versus  $18.13\pm 1.58\ \mu\text{m}$ ). All these values were also significantly different than controls. No differences were found in evaluating PF. At variance with the LAA, in CAF-MVD patients, the LAFW around the pulmonary vein ostia is a region characterized by a marked interstitial remodeling such that it may be morphologically indicated as an appropriate target for ablation treatment aimed at sinus rhythm restoration.

**Keywords** Atrial fibrillation · Fibrosis · Capillaries · Remodeling · Ablation

### Introduction

Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, is characterized by simultaneous reentrant wavelets with loss of organized atrial depolarization and efficient atrial contraction [1]. Its incidence increases with age and with associated structural heart diseases [16]. In fact, about 50% of patients undergoing mitral-valve surgery have chronic atrial fibrillation (CAF) [8]. However, the likelihood of sinus rhythm recovery after a conventional mitral-valve operation in the presence of CAF is less than 10% [15].

Ablation is a successful technique for restoring sinus rhythm in patients with chronic atrial fibrillation and structural heart disease. This approach is based on the assumption that without a large myocardial mass in which to operate, re-entrant circuits sufficient in number to perpetuate AF are unable to coexist [24]. Therefore, by causing lines of muscular tissue destruction, the purpose of this procedure is to reduce the myocardial atrial mass into areas electrically isolated from each other and too small to sustain AF. The swift development of several ablation methods using different energy sources to create lesions in different atrial locations, with or without left atrial appendage (LAA) exclusion, increased interest in the anatomic substrate that favors the onset and mainte-

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nance of the AF. Recent studies indicate that ablation of limited left atrial areas may be effective in the treatment of CAF associated with mitral-valve disease (CAF-MVD) [5, 9, 11, 21, 28]. In particular, the left atrial free wall near the pulmonary vein ostia (LAFW) seems to be a critical area for the maintenance of AF in patients with mitral-valve disease [28].

The aim of this study was to verify whether, in patients with CAF-MVD, this high-risk arrhythmogenic zone, located near the pulmonary veins, develops greater myocardial interstitial damage than a more remote left atrial area, such as the LAA.

## Materials and methods

A total number of 49 cases was studied. LAFW and LAA were sampled from 33 patients with CAF during combined mitral-valve surgery and radiofrequency ablation at the Cardiothoracic Surgery Unit of the S. Raffaele Hospital, Milan, Italy, and from 16 control autopsies performed at the Department of Pathology and Laboratory Medicine of the University of Parma, Parma, Italy. All specimens were collected during the period between February 2003 and June 2003.

### CAF-MVD patients

In conformity with clinical, echocardiographical [29] and Doppler criteria [22], 33 CAF-MVD patients were separated into three groups: 9 with mitral-valve stenosis (CAF-MVS), 13 with mitral-valve insufficiency (CAF-MVI) and 11 with mitral-valve stenosis/insufficiency (CAF-MVSI).

All patients underwent radiofrequency ablation and mitral-valve repair surgery as previously described in detail by Benussi et al. [5, 6]. Briefly, two encircling lesions around the ostia of the pulmonary veins were carried out epicardially, using a temperature-controlled multipolar radiofrequency catheter (Therma Line, Boston Scientific). After a conventional left atriotomy, the treatment was completed, with two ablative endocardial lesions connecting the two encirclings between them and connecting to the mitral-valve annulus. Subsequently, mitral-valve repair or replacement was performed, and the LAA was sutured.

The subjects enrolled in the present study signed an informed written consent to the procedure in accordance with a protocol approved by the Institutional Human Research Committee and the principles outlined in the Declaration of Helsinki.

### Control patients

All the autopsies were performed within 24 h after death, in accordance with ongoing Italian laws. The criteria for exclusion [23] from the control group were: medical record of any type of arrhythmia, atherosclerosis of the major coronary arteries with greater than 30% of reduction of the lumen, valvular abnormalities, acute or healed myocardial infarction, heart hypertrophy (>400 g for women and >450 g for men), neoplasms with metastasis, multiple myocardial focuses of the replacement fibrosis with a diameter greater than 2 mm, inflammatory cells in the myocardial interstitium, amyloidosis, sarcoidosis, chronic inflammatory processes of the respiratory system and connective tissue disorders.

### Tissue sampling for morphological and morphometric evaluations

Two left atrial samples were taken from each pathological and control heart: a strip of LAFW myocardium (adjacent to the atri-

otomy in the CAF-MVD patients) between the interatrial groove and the orifices of the left pulmonary veins and a portion of the LAA. The LAFW and LAA fragments measured 5×10 mm. After the excision, these specimens were fixed in 10% buffered formalin solution for 24–48 h and embedded in paraffin. Tissue blocks were sectioned at a thickness of 5  $\mu$ m and stained with hematoxylin-eosin and Van Gieson method for the collagen. Additional 5- $\mu$ m histological sections were stained with Picosirius Red (43665, Direct Red 80, Fluka, Buchs, CH) [26]. The Picosirius Red-stained sections were observed using a polarization light microscope to highlight the birefringence of the collagen fibers and, on the basis of the color, the type of collagen.

### Immunohistochemical methods

Immunohistochemical staining of the endothelial cells was performed on 5- $\mu$ m-thick paraffin-embedded sections using an anti-CD31 primary antibody (M0823, 1:20; Dako, Glostrup, Denmark). Briefly, sections were dewaxed in xylene, rehydrated through graded alcohols and xylene, and, after quenching of endogenous peroxidase with a 3% H<sub>2</sub>O<sub>2</sub> water solution, the specimens were incubated with a protein block (Ready to Use Dako Biotin Blocking System, Carpinteria, CA). Slides were then incubated with the primary antibody for 30 min. The reaction was revealed using, in order, the streptavidin-HRP Dako LSAB2 System, (K0675, Dako, Carpinteria, CA) and a 0.25% solution of 3–3'-diaminobenzidine tetrahydrochloride. Finally, histological sections were counterstained with Mayer hematoxylin, mounted and coverslipped.

### Ultrastructural examination

Ultrastructural examination was conducted in ten pathological and five control LAPW and LAA. A small myocardial fragment (2×1 mm) was cut out from the samples for the light microscopy analysis and immediately fixed in a mixture of 4% paraformaldehyde and 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 3 h at room temperature, post-fixed in 1% osmium tetroxide, dehydrated and embedded in araldite. A 1- $\mu$ m section was stained with methylene blue and safranin. Thin sections of selected fields were collected on a 300-mesh copper grid and, after staining with uranyl acetate and lead citrate, were qualitatively examined under a transmission electron microscope (Philips, EM 208S, Eindhoven, NL).

### Morphometric analysis

The percentages of myocardium occupied by myocytes and normal interstitium and replaced by interstitial fibrosis (IF) and perivascular fibrosis (PF) were evaluated using a light microscope (Olympus BX 51, Tokyo, Japan) with an ocular reticule containing 42 sampling points (No. 105844, Wild Heerbrugg Instruments, Inc.). Morphometric sampling was performed at a magnification of ×400 in Van Gieson method-stained histological sections and consisted of counting the fraction of ocular reticule points overlying the myocytes, the normal interstitium and the interstitial and perivascular sclerotic tissue (red color) [20]. Microscopic fields from the LAA ( $n=35$ ) and from the LAPW ( $n=35$ ) of each heart were measured.

Morphometric evaluation of the capillary density was performed on CD31-labeled tissue sections of 11 controls and 15 pathological atria. For each sample, 20 different histological images were taken, using a digital photcamera (Olympus DP11, Tokyo, Japan) at a magnification of ×400 and in fields in which cardiac muscle fibers and capillaries were transversely sectioned.

The capillary density (CD) per mm<sup>2</sup> of myocardium was calculated by counting the number of transverse capillary sections ( $N_c$ ) in the picture field area. Each image consisted of a square

tissue area equal to  $34,630 \mu\text{m}^2$ , measured using the Image-Pro Plus, 4.5 software (Media Cybernetics, Silver Spring, MD).

$$CD = N_c/0.03463$$

The capillary density per myocyte area ( $CD_m$ ) was obtained by relating the number of transverse capillary sections to the myocyte-cell tissue fraction ( $m\%$ ).

$$CD_m = (CD \cdot 100)/m\%$$

The tissue cylinder radius ( $R_t$ ), related to the maximal oxygen diffusion distance from the center of the capillary, was finally calculated using the Krogh's formula [17].

$$R_t = \sqrt{1/(\pi \times CD)}$$

### Statistical analysis

Data are expressed as mean±standard deviation. A normal distribution of the morphometric values was verified using the Kolmogorov-Smirnov test, and the differences among groups were assessed with a one-way analysis of variance and Student's *t* test for paired and unpaired data. Linear regression analysis between morphometric results and patient population and control data was carried out using the Pearson correlation coefficient. All tests were performed using SPSS version 10.0 software (SPSS Inc., Chicago, IL). *P* values less than 0.05 were considered as statistically significant.

## Results

### CAF-MVD patients

The patients enrolled in the study included 33 with CAF (9 males and 24 females), who were scheduled for mitral-valve surgery. They were characterized by 9 CAF-MVS (5 severe mitral stenosis and 4 severe mitral stenosis associated with mild mitral regurgitation), 13 CAF-MVI (severe mitral regurgitation) and 11 CAF-MVSI (5 severe mitral stenosis associated with moderate or severe mitral regurgitation, 6 moderate mitral stenosis associated with severe mitral regurgitation). Patient characteristics and main echocardiographical data are detailed in Table 1. Patients' ages and body surface areas were not statisti-

cally different among the three groups, and they were comparable with the controls. Of the patients, 23 (69.7%) had an underlying rheumatic disease and 10 (30.1%) had a degenerative mitral-valve disease.

Before heart surgery, 3 patients were in the New York Heart Association class I, 19 in class II and 11 in class III. CAF duration ranged from 6 months to 120 months (mean  $30 \pm 27$  months in the CAF-MVS,  $53 \pm 41$  months in the CAF-MVSI and  $57 \pm 50$  months in the CAF-MVI).

### Control patients

Control heart samples were collected from 16 autopsies: 7 males and 9 females. Ages varied from 40 years to 83 years, averaging  $61 \pm 15$  years; body surface areas ranged from  $1.58 \text{ m}^2$  to  $1.86 \text{ m}^2$  and averaged  $1.71 \pm 0.16 \text{ m}^2$ . Inclusion criteria in the control group were based on clinical, autoptical and histological data. Death in control patients was a consequence of cerebral hemorrhage (five cases), traumatic injury (four cases), septic shock (two cases), abdominal hemorrhage with hypovolemic shock (three cases), ketoacidotic coma (one case) and acute pyogenic meningitis (one case). Heart weight averaged  $388 \pm 41 \text{ g}$ . The macroscopic and histological examination of the other organs excluded all the pathologies listed in the exclusion criteria.

### Quantitative and qualitative fibrosis analysis

IF and PF percentages are detailed in Table 2. In pathological atria, the percentage of IF was significantly higher in LAFW samples than in the LAA ( $8.52 \pm 4.32$  versus  $3.03 \pm 1.71$ , respectively,  $P < 0.005$  in patients with CAF-MVS;  $6.75 \pm 2.95$  versus  $2.21 \pm 1.68$ ,  $P < 0.0005$  in CAF-MVSI and  $6.57 \pm 2.50$  versus  $2.42 \pm 0.83$ ,  $P < 0.0001$  in CAF-MVI). No differences were found among the three groups of patients. In addition, all pathological LAWV and LAA showed a conspicuous increase of IF compared with cor-

**Table 1** Patient characteristics and echocardiographic data. CAF chronic atrial fibrillation, CAF-MVS CAF with mitral-valve stenosis, CAF-MVI CAF with mitral-valve insufficiency, CAF-MVSI CAF with mitral-valve steno-insufficiency, CAF-MVD CAF with mitral-valve disease, LVEDD left ventricle end-diastolic diameter, LVESD left ventricle end-systolic diameter, LVEF left ventricle

ejection fraction, LA-SID left atrial superior-inferior dimension, LA-MLD left atrial medial-lateral dimension, LAA-NW left atrial appendage neck width, LAA left atrial appendage, PAPs pulmonary artery systolic pressure. Data are expressed as mean±standard deviation; range values are shown in parentheses

	CAF-MVS (n=9)	CAF-MVSI (n=11)	CAF-MVI (n=13)	Total CAF-MVD (n=33)
Age (years)	57±6 (45–64)	59±12 (34–75)	61±7 (50–72)	59±9 (34–75)
Sex, (male–female)	1–8	0–11	8–5	9–24
Body-surface area (m <sup>2</sup> )	1.70±0.15 (1.50–1.97)	1.62±0.15 (1.35–1.85)	1.78±0.18 (1.50–2.00)	1.70±0.17 (1.35–2.00)
CAF duration (months)	30±27 (6–84)	53±41 (6–120)	57±50 (4–120)	48±42 (6–120)
LVEDD (mm)	50±2 (47–53)	52±3 (47–58)	60±5 (50–70)	55±6 (47–70)
LVESD (mm)	34±5 (28–42)	34±6 (21–46)	38±8 (26–49)	36±7 (21–49)
LVEF (%)	58±7 (40–62)	59±7 (42–65)	57±8 (40–65)	58±7 (40–65)
LA-SID (mm)	71±9 (60–83)	60±14 (52–77)	63±9 (51–75)	66±10 (51–83)
LA-MLD (mm)	61±9 (46–72)	57±6 (53–59)	64±10 (53–82)	62±9 (46–82)
LAA-NW (mm)	22±2 (18–24)	25±4 (22–31)	24±5 (18–32)	23±4 (18–32)
LAA length (mm)	33±5 (26–40)	38±5 (34–44)	43±8 (30–50)	38±7 (26–50)
PAPs (mmHg)	48±12 (35–70)	49±10 (33–70)	46±15 (30–70)	48±12 (30–70)

**Table 2** Myocardial fibrosis. Data are expressed as mean±standard deviation; range values are shown in parenthesis. LAFW left atrial free wall around the pulmonary vein ostia, LAA left atrial appendage, CAF chronic atrial fibrillation, CAF-MVS CAF with mitral valve stenosis, CAF-MVI CAF with mitral-valve insufficiency, CAF-MVS CAF with mitral-valve steno-insufficiency, CAF-MVD CAF with mitral-valve disease

	CAF-MVS (n=9)			CAF-MVSI (n=11)			CAF-MVI (n=13)			Total CAF-MVD (n=33)			Controls (n=16)		
	LAA	LAFW	LAA	LAA	LAFW	LAA	LAA	LAFW	LAA	LAA	LAFW	LAA	LAA	LAFW	LAA
Interstitial Fibrosis (%)	3.03±1.71§ (0.64–6.53)	8.52±4.32*¶   (3.66–14.69)	2.21±1.68‡ (0.11–6.18)	2.21±1.68‡ (0.11–6.18)	6.75±2.95*# (3.19–12.40)	2.42±0.83‡ (1.12–3.88)	2.51±1.40* (0.11–6.53)	6.57±2.50†   (2.33–10.62)	7.16±3.23†   (2.33–14.69)	0.74±0.68 (0.00–2.32)	7.16±3.23†   (2.33–14.69)	0.74±0.68 (0.00–2.32)	0.74±0.68 (0.00–2.32)	1.23±0.75 (0.35–2.75)	0.74±0.68 (0.00–2.32)
Perivascular Fibrosis (%)	0.13±0.19 (0.00–0.60)	0.21±0.17 (0.00–0.48)	0.27±0.22 (0.00–0.55)	0.27±0.22 (0.00–0.55)	0.37±0.21 (0.08–0.74)	0.23±0.26 (0.00–0.80)	0.26±0.28 (0.00–1.00)	0.29±0.24 (0.00–0.81)	0.32±0.25 (0.00–0.97)	0.33±0.35 (0.00–1.00)	0.32±0.25 (0.00–0.97)	0.33±0.35 (0.00–1.00)	0.33±0.35 (0.00–1.00)	0.37±0.30 (0.08–0.97)	0.33±0.35 (0.00–1.00)

Statistical results. Pathological atria values compared with those of corresponding controls:

†=P<0.0001

\*=P<0.001

§=P<0.005

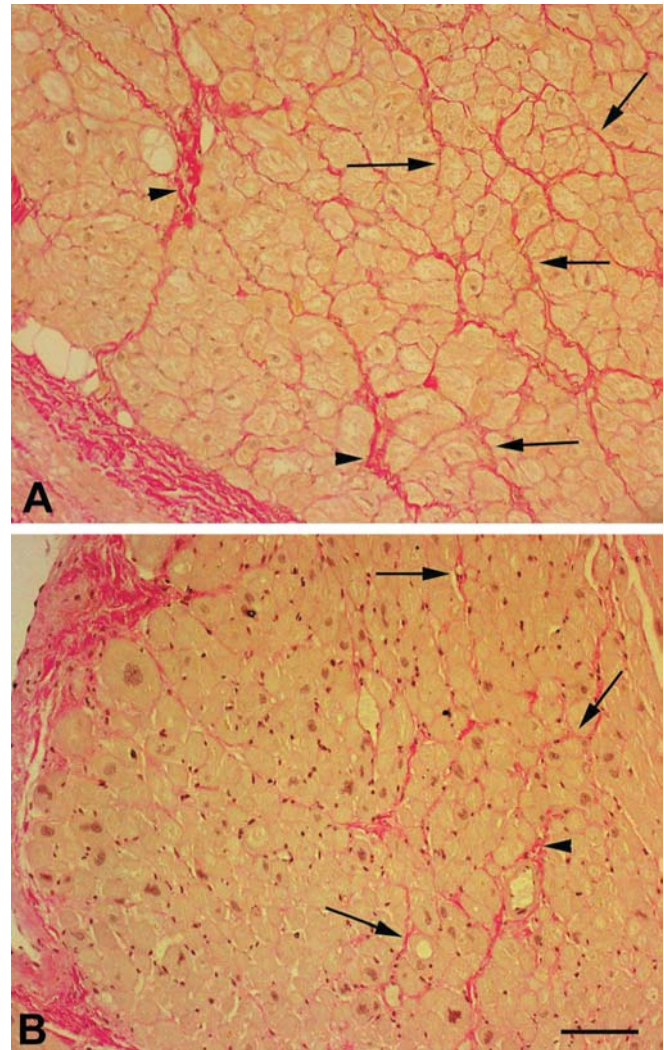
‡=P<0.05

LAFW values compared with those of LAA of the same group:

||=P<0.0001

#=P<0.0005

¶=P<0.005

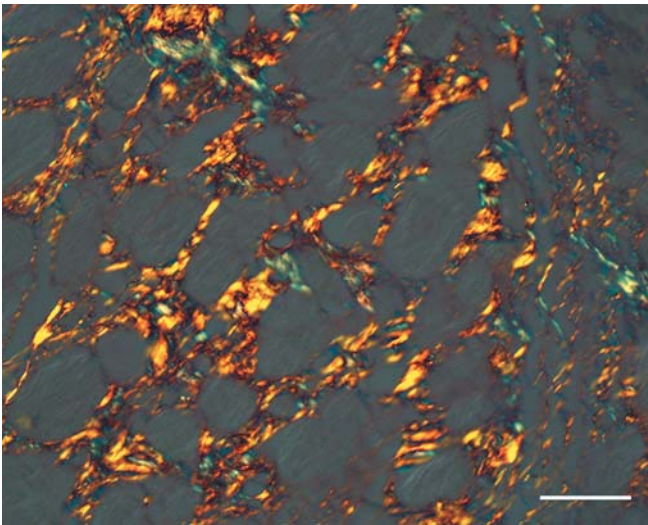


**Fig. 1** Photomicrographs from a patient with chronic atrial fibrillation associated with mitral disease. Histological appearance of the myocardial interstitial fibrosis (IF) (arrows) and the perivascular fibrosis (arrowheads) in the left atrial free wall around the pulmonary vein ostia (LAFW) (panel A) and left atrial appendage (LAA) (panel B). The LAFW shows a more intense IF than the correspondent LAA. Van Gieson stain. Bar is 100  $\mu$ m,  $\times$ 10

responding controls. In the control left atria, the percentage of IF was similar in the LAFW and the LAA (1.23±0.75 versus 0.74±0.68, respectively,  $P$ =n.s.). In pathological hearts, independently from groups, the LAFW and LAA IF percentages showed a statistically significant correlation (Pearson coefficient  $r=0.4$ ,  $P<0.01$ ).

No significant differences resulted from the comparison of the PF among the three pathological groups and the controls. Furthermore, no correlations were found between patients' age and CAF duration when related to the percentages of IF and PF and to the echocardiographical measurements.

All histological samples were examined using hematoxylin and eosin staining to confirm the sample preservation and the Van Gieson method for collagen to reveal IF and PF (Fig. 1). Interstitial foci of fibrosis sur-



**Fig. 2** Left atrial free wall around the pulmonary vein ostia histological appearance in a patient with chronic atrial fibrillation associated with mitral disease. The atrial myocytes are diffusely surrounded by a marked collagen network (orange stain). Picosirius Red stain, observed by polarization light microscopy. Bar is 30  $\mu\text{m}$ ,  $\times 40$

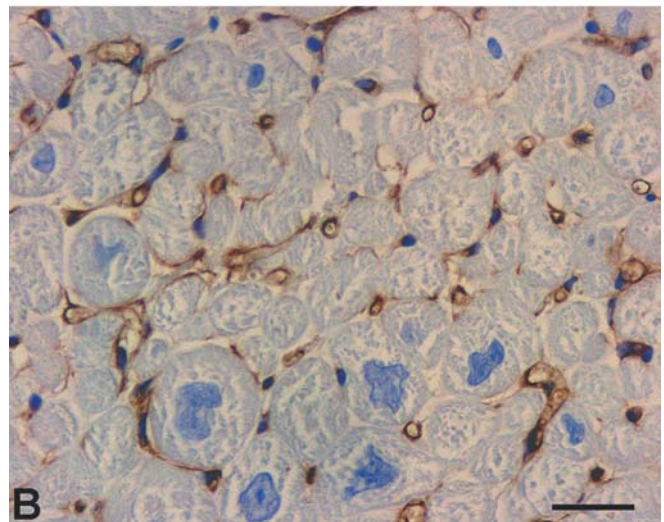
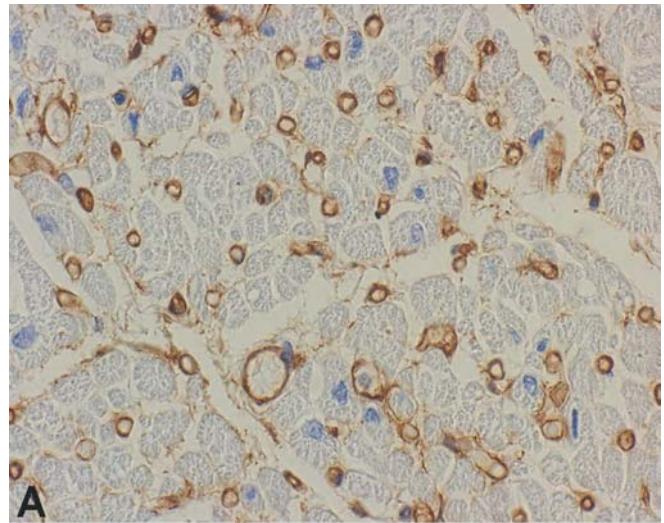
rounded the myocytes in a fine network that often distorted the architecture of the tissue. Some samples, in which the IF was more evident, also showed a conspicuous thickening of the subendocardial soft tissues. Furthermore, polarization light microscopy on Picosirius Red-stained sections suggested the presence, as orange color, of type-I collagen in the interstitial, perivascular or subendocardial sclerotic tissue (Fig. 2).

At discharge, 29 patients (87.9%) were in stable sinus rhythm, while in 4 (12.1%, 1 with mitral-valve stenosis and 3 with mitral-valve insufficiency), AF recurred. These 4 patients showed an LAFW IF far greater than the average value of their group: 14.69% with respect to an average of 8.52% in the CAF-MVS; 8.20%, 10.62% and 9.88% with respect to an average of 6.57% in the CAF-MVI. In addition, 2 of these CAF-MVI subjects showed a percentage of LAA IF more extensive than the average value of its group (3.02% and 3.56% with respect to an average of 2.42%).

#### Quantitative capillary network analysis

Endothelial cells were immunohistochemically revealed using an anti-CD31 primary antibody (Fig. 3). Quantitative capillary network analysis was performed in 11 controls and in 15 pathological left atria (3 with CAF-MVS, 4 with CAF-MVI and 8 with CAF-MVSI) and without separation into groups. Control atria showed a higher capillary density and a more regular microvascular architecture than the pathological hearts.

Capillary network characteristics expressed as myocardial capillary density, capillary density related to the myocyte area and the maximal oxygen diffusion distance



**Fig. 3** Photomicrographs from left atrial free wall around the pulmonary vein ostia. Immunohistochemical staining (brown color) for CD31 endothelial antigen in a control (panel A) and in a patient with chronic atrial fibrillation associated with mitral disease (panel B) showing a significantly lower capillary density in the pathological subject. Counterstained with Mayer hematoxylin. Bar is 30  $\mu\text{m}$ ,  $\times 40$

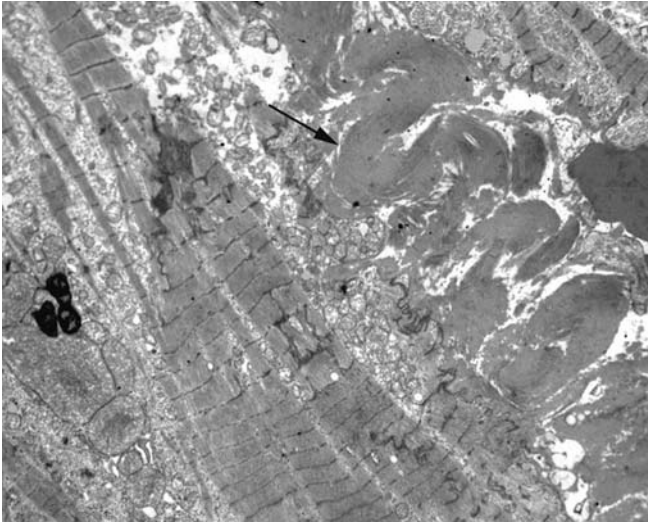
[17, 19] (Krogh's tissue cylinder radius,  $R_t$ ) are listed in Table 3. CAF-MVD atria showed a significant reduction of the myocardial capillary density ( $-29.0\%$ ,  $P < 0.0001$  in the LAA and  $-39.8\%$ ,  $P < 0.0001$  in the LAFW) and of the capillary density corrected for the myocyte area ( $-32.3\%$ ,  $P < 0.0001$  in the LAA and  $-40.9\%$ ,  $P < 0.0001$  in the LAFW), compared with controls. In parallel, the maximal oxygen diffusion distance increased in pathological atria ( $+15.9\%$ ,  $P < 0.0001$  in the LAA and  $+22.7\%$ ,  $P < 0.0001$  in the LAFW). In pathological LAFW, the myocardial capillary density and the oxygen maximal diffusion distance were statistically different when compared with the LAA ( $-16.1\%$ ,  $P < 0.05$  and  $+8.0\%$ ,  $P < 0.05$  respectively, in the LAFW). In control atria, no differences were found between LAA and LAFW capillary network parameters.

**Table 3** Myocardial capillary network. Data are expressed as mean±standard deviation; range values are shown in parenthesis. LAFW left atrial free wall around the pulmonary vein ostia. LAA left atrial appendage, CAF-MVD chronic atrial fibrillation with mitral-valve disease

	Total CAF-MVD (n=15)		Controls (n=11)	
	LAA	LAFW	LAA	LAFW
Capillary density (no./mm <sup>2</sup> of myocardium)	989±173* (736–1291)	830±106*† (670–1020)	1392±107 (1215–1522)	1379±57 (1276–1459)
Capillary density per myocyte area (no./mm <sup>2</sup> of myocytes)	1052±179* (785–1383)	951±123* (8788–1201)	1554±140 (1329–1707)	1609±89 (1474–1714)
Maximal O <sub>2</sub> diffusion distance (μm)	18.13±1.58* (15.71–20.80)	19.70±1.27*† (17.67–21.81)	15.16±0.60 (14.47–16.19)	15.21±0.32 (14.78–15.80)

\* $P < 0.0001$  compared with corresponding control

† $P < 0.05$  compared with CAF-MVD LAA



**Fig. 4** Ultrastructural appearance of the left atrial free wall around the pulmonary vein ostia interstitial fibrosis in a patient with chronic atrial fibrillation associated with mitral disease. Bands of collagen (arrow) between two myocytes. Original magnification ×2800

#### Ultrastructural examination

The myocardial collagen deposition and the distortion of the intercellular space of the CAF-MVD specimens were confirmed using ultrastructural analysis. Among myocytes showing characteristics of cellular dedifferentiation [2], bands of collagen enlarged the interstitial spaces, thus modifying the normal myocardial architecture (Fig. 4). Fibroblasts and adipocytes were found in some IF areas, particularly near the blood vessels. The extent of the interstitial damage was always greater in the LAFW than in the LAA specimens.

#### Discussion

The present study shows that CAF-MVD patients present several modifications in the atrial interstitium with significant differences between LAFW and LAA. In the LAFW, this remodeling was mainly characterized by a higher degree of IF and a lower capillary density.

The CAF-MVD patients showed, on average, an IF of 7.16% in the LAFW and 2.51% in the LAA (2.85-times greater), without differences among the three pathological groups. No statistical differences for PF were found in examining pathological and control atria.

IF plays a role as a reparative process and can interfere with myocardial electrical activity. In the 1980s, Boyden et al. [7] studied the structure of left atrial enlargement and its effects on electrophysiological properties in dogs with mitral-valve disease. Extensive IF was found in the atria between enlarged myocytes. These atria were highly susceptible to the initiation and perpetuation of atrial arrhythmias, while the resting and action potentials did not differ from the non-enlarged right atrium or in the control atria. In fact, atrial dilation would permit the co-existence of many re-entrant circuits, and structural interstitial remodeling would alter anisotropic properties (leading to slow, zig-zag conduction, unidirectional block) and would also increase the dispersion of refractoriness favoring re-entrant circuits [2, 18, 27].

Furthermore, in atria with severe IF, sympathetic and parasympathetic fibers may be interrupted, causing regional supersensitivity to catecholamines and acetylcholine and dishomogeneities in refractoriness [14]. Thus, a highly sclerotic region can be a local source for AF.

In our patient population, the recurrence, after radiofrequency treatment, of AF in four subjects with extensive LAFW IF supports the role of the myocardiosclerosis as a structural factor in the pathogenesis of the AF in subjects with MVD. In these patients, a great amount of IF, usually found around the pulmonary vein ostia, could also be present in the atrial myocardium interposed between LAPW and LAA, thereby explaining the ineffectiveness of the ablative approach. In fact, two patients also showed a noteworthy IF in the LAA myocardium.

IF percentages in LAA and LAFW were different and statistically correlated. An explanation of this asymmetric and parallel myocardial injury could be the close anatomical position of the investigated atrial area to the pulmonary vein orifices, a region subjected to an intense stress. Furthermore, the fact that the internal diameter of the left atrium is greater than the LAA diameter [12, 30]—even though the internal left atrial and LAA pressures are similar—determines a higher tension in the atrial free wall than in the LAA (according to Laplace's

law). In addition, considering that the LAFW and the LAA have different embryological origins—the trabecular LAA is the only remnant part of the original embryonic left atrium, while the left atrial smooth wall develops from the outgrowth of the pulmonary veins—we suggest that a different embryological origin of the two areas may contribute to a different reaction to stress factors [25].

Differences in regional distensibility and function exist between the left atrial body and the LAA. The LAA appears more compliant than the remaining left atrium, and it may play a crucial role in maintaining hemodynamic function when filling pressures are elevated [10, 13]. Extracellular matrix remodeling of the left atrium in patients with hemodynamically overloaded and with dilated atria, favors the occurrence and the perpetuation of AF as well as the reduction of left atrial compliance. Therefore, in the light of our results, differences in regional distensibility and function existing between LAFW and LAA may highlight the role of the LAA in maintaining the hemodynamic function in patients with elevated filling pressures.

In our study, we evaluated three microvascular morphological parameters, within the myocardial interstitial remodeling: the myocardial capillary density, the myocardial capillary density related to the myocyte area and the maximal oxygen diffusion distance. These parameters were significantly different than the controls. In the interstitial remodeling, capillary densities and maximal oxygen diffusion distance, not being statistically correlated with the IF and PF percentages, behaved as independent variables. Myocyte hypertrophy, myocyte-cell loss with reparative fibrosis and stretching-induced atrial enlargement could induce a reduction of the capillary density as well as an increase in the maximal oxygen diffusion distance [4] (+15.9% in the LAA and +22.7% in the LAFW). Additionally, the maximal oxygen diffusion distance and the myocardial capillary density were more altered in the LAFW when compared with the LAA in CAF-MVD patients (+8.0% and -16.1%, respectively, in the LAFW). These modifications can expose the myocardium to a higher risk of damage in cases of increased need for oxygen [3, 4]. Thus, we suggest that this smaller capillary network may contribute to sustaining arrhythmogenic focuses.

In conclusion, in patients with CAF-MVD, the interstitial remodeling process is quantitatively different in the LAFW than in LAA. The LAFW myocardium, located near the pulmonary vein ostia, shows a higher percentage of IF and a reduced capillary density. These features could represent an aspect of the anatomic substrate for the CAF in this subset of patients and may actually have some implications regarding the treatment strategy. The kind of interstitial damage occurring in the perivenous LAFW indicates this anatomic area as an appropriate target for ablations aimed at restoring sinus rhythm in patients with CAF-MVD. However, based on the finding that the LAA appears to be more heavily protected in interstitial remodeling, systematic LAA exclusion in patients with AF and mitral-valve disease who are undergoing surgery should be reconsidered.

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