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# Early left atrial tissue features in patients with chronic mitral regurgitation and sinus rhythm: Alterations of not remodeled left atria☆

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ABSTRACT

*Objective:* Left atrial (LA) enlargement, a compensatory mechanism in chronic mitral regurgitation (MR) increasing the risk of atrial fibrillation (AF) and predictive of cardiac events, involves structural alterations. We characterized LA features in patients in sinus rhythm with severe degree of MR, similar degrees of left ventricular remodeling but divergent LA size.

*Methods:* Among a consecutive series of 163 patients in stable sinus rhythm undergoing isolated mitral valve surgery for severe non-rheumatic MR, two groups were arbitrarily selected according to their LA size (antero-posterior): NRLA group (non-remodeled LA) included 8 patients with LA ≤ 40 mm, RLA group (remodeled LA) included 8 patients with LA > 55 mm. LA biopsies were processed for paraffin inclusion and sectioning. Fibrosis, cardiomyocytes morphology, capillaries density, cytochrome c and F-actin expression were evaluated by microscopy.

*Results:* Histology and immunohistochemistry demonstrated alteration of moderate entity: higher amounts of endomysial fibrosis (not of collagen type III) and of hypertrophic cardiomyocytes in RLA than in NRLA. Confocal microscopy displayed focally disorganized F-actin and no nuclear fragmentation in both groups, but more intra-cytoplasm cytochrome c in RLA vs. NRLA, possibly indicative of more successful escape to apoptosis by NRLA cardiomyocytes.

*Conclusions:* Our study shows the presence of early cellular and interstitial alterations in LA tissue in patients with chronic MR and sinus rhythm. These features were analogous to those of patients with AF, and suggest that macroscopic remodeling LA in the settings of MR is preceded by structural changes, paving the way to further investigation on the preventive role of early mitral valve repair.



## 1. Introduction

Chronic mitral regurgitation (MR) is a frequent cause of left atrial (LA) dilatation, as the result of volume overload. The increase of the LA size is a compensatory mechanism allowing accommodation of the regurgitant volume at a lower filling pressure and reducing the symptoms of pulmonary congestion [1]. The degree of LA dilatation is variable, and usually is related to the degree of MR and to the duration of the disease [2–4].

Besides its compensatory nature, in patients with structural heart disease LA dilatation is a predictor of clinical events such as the development of congestive heart failure, atrial fibrillation (AF), and increased thromboembolic risk [5,6]. In the specific setting of patients with chronic MR, the presence of LA dilatation has been associated with a higher chance of developing congestive heart failure [7]. Additionally, the risk of developing AF in patients with chronic MR in sinus rhythm at the time of diagnosis, is the highest in the presence of LA dilatation [8]. Structural abnormalities of dilated atria contribute to the initiation and sustainment of AF in patients with mitral valve disease [9–11]. However little is known on the structure of not dilated LA in patients with severe chronic MR and in sinus rhythm.

This study has been conducted to identify the structural changes in the LA wall of patients with chronic MR in sinus rhythm, in the absence of previous history of AF, with and without LA dilatation, but with similar degrees of left ventricular remodeling.

☆ Francesco Maisano, Ottavio Alfieri, Gabriele Fragasso and Chiara Foglieni take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation. Raffaella Rusconi and Maria Elena Mantione realized the experimental study on LA biopsies.

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## 2. Methods

### 2.1. Study population

Patients in sinus rhythm (n = 163) undergoing isolated mitral valve surgery for severe non-rheumatic chronic MR were selected. In all patients MR was due to either chordal rupture or prolapse/flail of one or both leaflets, due to myxomatous and fibroelastic degeneration. Subjects with positive history of atrial arrhythmias, symptomatic palpitations, other cardiac diseases, diabetes or renal diseases were excluded from study. Before surgery, all patients were screened with ECG Holter monitoring to exclude the occurrence of asymptomatic arrhythmias. Patient's clinical characteristics are listed in Table 1.

Patients underwent routine trans-esophageal echocardiography just before surgery. The trans-esophageal examination was conducted in the awake state, under local anesthesia and mild sedation. All patients had severe degree of MR, graded with quantitative measurements using at least one of the following quantitative methods: (i) proximal isovelocity surface area (PISA) analyzed by the proximal flow convergence; or (ii) vena contracta width. MR was classified as mild (effective regurgitant orifice area, EROA ≥0.10 < 0.20 $cm^2$, vena contracta width ≥ 0.2 < 0.3 cm), moderate (EROA ≥0.20 ≤ 0.39 $cm^2$, vena contracta width ≥ 0.3 < 0.69 cm), or severe (EROA ≥0.40 $cm^2$, vena contracta width ≥ 0.69 cm) [12]. The antero-posterior dimension of the left atrium was measured in the parasternal long axis view. According to echocardiographic findings, the study population was recruited as a subgroup of patients with similar degrees of left ventricular remodeling, but divergent LA dimensions: a homogeneous group of patients within ± 1SD from the mean of the left ventricular end-diastolic diameter (LVEDD) was extracted from the overall population. These patients had a mean LVEDD of 57.5 mm ± 6.9 mm (range 51 mm–64 mm). Within this cohort of patients, an arbitrary category classification was applied, with the only purpose to evidence two opposite extreme degrees of LA size. Two groups were selected according to their LA size (antero-posterior): the NRLA group (non-remodeled left atrium) included 8 patients with a normal size LA (LA ≤ 40 mm), the RLA group (remodeled left atrium) included 8 patients with LA > 55 mm. These cut-off values corresponded to ± 1SD from the mean of LA dimensions in the entire subpopulation; patients in group NRLA had LA dimensions lower than − 1SD from the mean and RLA patients had LA dimensions + 1SD from the mean, but all patients had similar degree of left ventricular remodeling.

**Table 1**
Clinical characteristics of NRLA (non-remodeled left atrium) group vs. RLA (remodeled left atrium) group.

| | RLA (n = 8) | NRLA (n = 8) | P |
|---|---|---|---|
| Age (years) | 61.1 ± 9.12 | 63.6 ± 9.24 | 0.59 |
| Gender (M/F) | 8/0 | 7/1 | 0.23 |
| Height (cm) | 177.3 ± 6.39 | 174.0 ± 10.24 | 0.48 |
| Weight (kg) | 78.1 ± 7.03 | 74.8 ± 7.33 | 0.41 |
| Hypertension (y/n) | 6/2 | 7/1 | 0.73 |
| NYHA class (I/II/III/IV) | 2/4/2 | 4/4/0 | 0.17 |
| Time elapsed from diagnosis (years) | 21.7 ± 18.9 | 3.14 ± 3.6 | 0.02* |
| LVEDD (mm) | 59.6 ± 2.72 | 58.0 ± 2.13 | 0.2 |
| LVESD (mm) | 36.4 ± 4.64 | 34.6 ± 4.47 | 0.46 |
| LVEDV (ml) | 154.7 ± 29.4 | 134.2 ± 20.46 | 0.13 |
| LVESV (ml) | 53.5 ± 13.6 | 49.6 ± 7.6 | 0.49 |
| Stroke volume (ml) | 101.2 ± 28.21 | 84.6 ± 15.1 | 0.16 |
| EF (%) | 64.8 ± 8.32 | 62.8 ± 4.06 | 0.54 |
| LVED long axis (mm) | 82.0 ± 9.81 | 65.4 ± 26.38 | 0.17 |
| LVED short axis (mm) | 67.7 ± 9.3 | 55.8 ± 12.95 | 0.11 |
| Septal thickness (mm) | 12.2 ± 1.67 | 12.1 ± 1.81 | 0.88 |
| Posterior wall thickness (mm) | 10.4 ± 1.13 | 11.0 ± 1.41 | 0.42 |

LVEDD = Left Ventricular End Diastolic Diameter, LVESD = Left Ventricular End Sistolic Diameter; EF = Ejection Fraction, LVED = Left Ventricular End Diastolic.

Patients with NRLA had a significantly shorter clinical history, with delay from diagnosis to surgery being 3.0 ± 3.6 years vs. 21.0 ± 18.9 years in patients with RLA (p = 0.02). All other clinical and functional parameters were comparable. LA diameter was 40 ± 0.9 mm in the NRLA group as compared to 57 ± 0.9 mm in the RLA group (p < 0.0001). Table 1 summarizes patient's clinical data.

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the internal review board of the ethic committee at San Raffaele Hospital and all patients signed an informed consent.

All patients underwent open-heart mitral valve repair surgery, conducted under general anesthesia and mildly hypothermic cardiopulmonary by-pass. Myocardial protection was accomplished by means of anterograde and retrograde cold blood cardioplegia. Following aortic cross-clamping and cardioplegia administration, the LA was opened as routine adjacent to the interatrial groove. A full thickness stripe of LA tissue was taken from one margin of the atriotomy, in a region close to the right inferior pulmonary veins, but always in the body of the LA. Average dimension of the biopsies measured 2 × 4 × 10 $mm^3$.

### 2.2. Tissue processing

The specimens were fixed after excision in 10% buffered formalin, processed for paraffin inclusion and serially sectioned (5 μm thick). Dewaxed sections were submitted either to hematoxylin/eosin, Movat's pentachrome, Janus Blue (a metachromatic dye specifically binding DNA and glycogen) [13]. Cytomyolysis, myofibrillar abnormalities, lipofuscin and glycogen granules in cardiomyocytes, and inflammatory cells presence were evaluated under Eclipse 55i microscope equipped with a DS-L1 camera (Nikon, Tokyo, Japan).

After microwave antigen retrieval, additional sections were submitted to immunofluorescence or immunohistochemistry to detect endothelial cells (von Willebrand factor, vWF), collagen type III, proliferating cells (ki67), or mitochondria cytochrome c (Cyt c), revealed by donkey-anti-rabbit or rabbit anti-mouse IgG-AlexaFluor 488 (Molecular Probes, Eugene, OR) (1:500 in DPBS, 45 min RT) or by ABC method (ABC élite, Vector, Burlingame, CA). F-actin cytoskeleton was labeled by FITC-conjugated Phalloidin (50 U/ml, 60 min, RT); nuclei by 4,6-Diaminidino-2-Phenyindole (DAPI, 0.2 nM, 20 min, RT) (Sigma-Aldrich, Milano, Italy).

### 2.3. Morphometry

The muscle area intra-perimysium (MA, occupied by cardiomyocytes, endomysial connective and capillaries) and the density of cardiomyocytes (out of 2000 total cells/sample) were measured in a mean total area (TotA) of 4.12 ± 1.64 $mm^2$/patient. The interstitial area (IntA = TotA-MA), the ratio MA/TotA and the ratio IntA/TotA were calculated.

Endomysial fibrosis was determined in a mean MA/patient of 0.64 ± 0.43 $mm^2$. Since Movat's allowed distinguish reticular fibers with a high content of collagen from fibers with a prevalence of proteoglycans, the relative contribute of both components to endomysial fibrosis was also evaluated. All analyses were performed by objective 20×. The collagen type III content into endomysial (Coll intra) and interstitial (Coll extra) compartments was measured (objective 40×) in a mean tissue fraction of 0.52 ± 0.12 $mm^2$.

An average of 350 capillaries/patient in a tissue fraction of 1.40 ± 0.60 $mm^2$ was counted in vWF labeled sections (objective 20×) and the density of endomysial capillaries in cardiomyocytes area (Cdm) was calculated: Cdm = (100*number of capillaries/cardiomyocytes area)%. The ratio between capillaries number/cardiomyocytes number inside the tissue fraction (Capn/Cardn) was determined.

Major and minor diameters of transversally sectioned cardiomyocytes were measured (objective 20×) in 6 fields/patient, and cardiomyocyte areas calculated by approximating the cell shape to an