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Histopathological Substrate For Chronic Atrial Fibrillation in Humans

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Abstract

Background—There is a lack of understanding of the substrate for microreentrant circuits and triggered activity of the pulmonary vein (PV) muscle sleeves and atria, in patients with atrial fibrillation (AF).

Objectives—To examine the histological substrate of patients with chronic AF.

Methods—We stained 23 biopsies taken from the PV-LA junction and RAA from 5 chronic AF patients and 3 sinus rhythm (SR) patients undergoing mitral valve surgery using periodic acid-Schiff (PAS), and antibodies to hyperpolarization-activated cyclic nucleotide-gated potassium channel 4 (HCN4), CD117/c-kit, myoglobin, tyrosine hydroxylase (TH), growth-associated protein 43, cholineacetyltransferase, and synaptophysin, as well as trichrome.

Results—As opposed to being clustered together in the subendocardial layer in SR patients, PAS-positive cells were separated from each other by inflammatory infiltrate and collagen fibers in AF patients. These cells stained positively for HCN4 and myoglobin, indicating they were cardiomyocytes that might have a potential pacemaking function, but different from CD117/c-kit-positive interstitial Cajal-like cells (ICLC). In AF patients, the intercellular space was occupied by a lymphomononuclear infiltrate (100% vs. 33% in SR patients, $p=0.002$), and a greater amount of interstitial fibrosis ($37\pm5.6\%$ vs. $7.4\pm2.8\%$, $p=0.009$). Nerve densities did not differ between AF and SR patients. However, the density of sympathetic nerve twigs in AF patients was significantly greater as compared to the others nerves ($p=0.03$).

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Conclusions—HCN4-/PAS-positive cardiomyocytes, and CD117/c-kit-positive ICLC scattered among abundant inflammatory infiltrate, fibrous tissue, and sympathetic nerve structures in the atria and at the PV-LA junctions might be a substrate for the maintenance of chronic AF.

Keywords

Atrial fibrillation; Electrophysiology; Pathology; Remodeling; Substrate

Introduction

There continues to be a lack of understanding of the pathogenesis of atrial fibrillation (AF). It has been shown that structural remodeling associated with aging and heart disease, such as increased myocardial fibrosis (1,2), plays an important role in AF. Heightened atrial sympathetic innervation has been demonstrated in patients with persistent AF (3), as has been the presence of cells that stain with periodic-acid Schiff (PAS) (4), and interstitial Cajal-like cells (ICLC) in the human PV muscle sleeves, that might act as triggers (5,6). Abundant PAS-positive cells were found at the site of focal discharges in the canine PV-left atrium (PV-LA) junction preparations (4), raising the speculation that these cells might serve as a source of automaticity. However, the nature and function of PAS-positive cells has not been assessed. The present study was designed to investigate the underlying histopathological differences between AF and sinus rhythm (SR) patients, and gain further knowledge about AF substrate. Intraoperative myocardial biopsies of live tissue were taken from selected areas at the PV-LA junctions, and at the RAA. Tissue specimens were examined for the presence and nature of PAS-positive cells, ICLC, inflammatory infiltrate, fibrosis, as well as other markers of neural and structural remodeling.

Methods

Patient Characteristics

From August 2006 to February 2007, 8 patients, 6 of them women, (mean age 70.9 ± 12.1 years), undergoing mitral valve surgery were prospectively enrolled in the study. The research protocol was approved by the Institutional Review Board of the Hackensack University Medical Center and written informed consent was obtained from all patients. The specimens, without patient identifier information, were shipped to Cedars-Sinai Medical Center, University of California Los Angeles, and the Krannert Institute of Cardiology, Indiana University for histopathological analyses in compliance with Occupational Safety and Health Administration guidelines. The mean LA diameter was 51.3 ± 7.1 mm, and 7 patients had heart failure with New York Heart Association functional classes III–IV. Five patients were in AF persistently for a mean of 58.8 ± 51.33 months, and refractory to ≥ 2 drug regimens. Three patients were in SR. Details are summarized in Table 1.

Clinical Assessment

Pre-operative atrial rhythm was established by 24-hour electrocardiogram Holter monitoring in the preceding months. All patients received routine pre-operative evaluation including electrocardiogram, echocardiography, and coronary angiography, without complications.

Intraoperative Myocardial Biopsy and Tissue Processing

Mitral valve surgery was performed using standard video-assisted approach through a right mini-thoracotomy. Specimens consisting of the entire thickness of the atrial wall were excised. A total of 23 myocardial specimens (2.9 ± 0.4 specimens per patient, $4 \times 4 \times 2$ mm sized) were obtained from the right superior (RS) PV-LA junction ($n=8$), the right inferior (RI) PV-LA junction ($n=8$), and the right atrial appendage (RAA) ($n=7$) as shown in Figure 1. All

myocardial specimens were immediately fixed in Carnoy's solution for PAS-staining and in 10% formalin solution. Tissues were embedded into paraffin blocks, and routinely processed for histological studies.

Histology and Immunohistochemistry

Multiple 5- μ m thick serial sections were used. PAS (Accustain Schiff's reagent, Sigma-Aldrich) staining was performed to assess intracellular glycogen content. The PAS-positive cellular material was treated with diastase, to confirm its disappearance. Serial PAS stained sections were processed with an anti-myoglobin antibody (1:500 dilution, rabbit anti-human polyclonal; Affinity BioReagents), anti-hyperpolarization-activated cyclic nucleotide-gated potassium channel 4 (HCN4, 1:200 dilution, Chemicon, AB5808), and polyclonal CD117/c-kit (1:100 dilution, A4502, DAKO Cytomation). Masson's trichrome, and hematoxylin-eosin stains were used to determine the presence and degree of fibrosis, and inflammatory infiltrates. Immunostaining by the avidin-biotin complex method was performed using antibodies for tyrosine hydroxylase (TH, 1:100 dilution, Accurate Chemical, BYA90291), growth-associated protein 43 (GAP43, 1:50 dilution, Chemicon, AB5312), choline acetyltransferase (ChAT, 1:50 dilution, Chemicon, AB144P), and synaptophysin (SYN, 1:50 dilution, Chemicon, 04-353) to label adrenergic nerves twigs, neural growth protein expressed in sprouting axons, cholinergic nerves twigs, and neurotransmitter exocytosis, respectively, as previously described (7). The tissues from AF and SR patients were processed in the same session. The slides were examined by light microscopy.

Analysis of Structural Anisotropy and Nerve Density Determination

A computer-assisted histomorphometry analyzer (MagicWand) was used to assess the amount of fibrosis, expressed as percentage of the total area. The boundary delimitations between the physiological collagen fibers and the pathological fraction of sclerotic tissue were manually ensured to avoid an overestimation of interstitial fibrosis. The computer automatically detected the stained nerves by their brown color on the slide using 40x magnification. It then calculated the number and area divided by the total area examined. Nerve densities were calculated and corrected by the amount of fibrosis using the formula: density/(100 – percentage of collagen content).

Analysis of PAS-positive Cells and Interstitial Cajal-like Cells

Histomorphometry of PAS-positive cells included the evaluation of presence, location, nature, morphology, and size of PAS-positive cells using 10x magnification as previously described (4). The transverse thickness of PAS-positive-cell layers was measured using a slide micrometer (Leica, 2 mm div. into units of 0.01 mm). The presence and location of HCN4- and anti-myoglobin- positivity was assessed by light microscopy. The presence of CD117/c-kit-positive ICLC was assessed by light microscopy identifying their morphological and distribution characteristics, such as long and thin cellular processes and intercellular location, as previously described (5,6).

All analyses were performed by a pathologist blinded to clinical information in up to 4 representative fields with greater staining for each slide.

Statistical Analysis

Data obtained from the computerized histomorphometry system were analyzed as previously reported (4). Continuous variables were expressed as mean $\pm$ SD. Student's *t* tests for paired or unpaired determinations were used to compare the means of 2 groups for continuous variables. Comparison of categorical variables was performed with a χ^2 test. Analyses of

variance (ANOVA) compared the means among 3 or more groups. A p value ≤ 0.05 was considered statistically significant.

Results

AF patients were significantly older (77.4 ± 10.5 vs. 60 ± 2.6 , $p=0.03$), and had a larger LA (55.6 ± 4.6 vs. 44 ± 3 , $p=0.009$) than SR patients (Table 1).

Histology and Immunohistochemistry

Table 2 summarizes the histological and immunohistochemical findings of the study. Table 3 compares the results between PV-LA junction and RAA samples of AF and SR patients to assess the preferential locations of the significant findings.

Distribution of PAS-Positive Cells and Interstitial Cajal-like Cells

PAS-positive cells were located in the subendocardial layer in both AF and SR patients, with a unique pattern of distribution. The cellular glycogen content of the PAS-positive cells (Figure 2A) was demonstrated by its digestion and removal by diastase treatment (Figure 2B). PAS- (Figures 2C and 2E, respectively), anti-myoglobin- (Figure 2D) and HCN4- (Figure 2F) positive stainings were colocalized, showing that PAS-positive cells are cardiomyocytes that might have a potential pacemaking function. ICLC with thin cellular processes were located in the interstitium close to nerve bundles, blood vessels, and atrial and PV sleeve myocytes (Figures 2G and 2H). In SR patients, PAS-positive cardiomyocytes were clustered together (Figure 3A), separated by minimal interstitial collagen (Figure 3B) at the PV muscle sleeve. In patients with chronic AF, however, PAS-positive cardiomyocytes were separated (Figure 3C) by abundant inflammatory infiltrate (described below) and collagen fibers (Figure 3D) at the PV muscle sleeve. Figure 4 illustrates another example of an AF patient with similar findings at the RAA (Panels A, B and C) and the PV muscle sleeve (Panels D, E and F). The transverse size of clustered or non-clustered PAS-positive cells and thickness of PAS-positive cell layers did not differ between AF and SR patients ($p=ns$, Table 2). Non-clustered PAS-positive cardiomyocytes were seen in the PV-LA junctions (7/10 specimens) as well as RAA (4/5 specimens) of AF patients but not in SR patients (0 specimens, $p=0.01$, Table 3).

Inflammatory Infiltrate and Fibrosis

Clusters of chronic lymphomononuclear inflammatory infiltrate were associated with pronounced vacuolar degeneration of the adjacent atrial myocytes (Figure 5A), and extensive patchy fibrosis (Figure 5B) in all AF patients, and were present in only 1 SR patient ($p=0.002$, Table 2). The infiltrate was equally distributed between the PV-LA junction and the RAA sites ($p=ns$) in AF patients. This infiltrate was however more frequently observed in the PV-LA junction sites of AF vs. SR patients (100% vs. 33.3%, $p=0.0082$, respectively, Table 3). The mean amount of fibrosis in AF patients was significantly greater as compared to SR patients ($p=0.009$, Table 2), although this was similarly distributed between the PV-LA junction and RAA ($p=ns$, Table 3).

Distribution of Sympathetic Nerve Endings

AF patients had abundant sympathetic nerve structures located in the border zones between fibrosis and viable myocytes, as well as in the perivascular area. In the central area of fibrosis, these structures were difficult to find. TH-immunostained sympathetic nerve density was higher in AF patients than SR patients, but not statistically significant (Figure 4G). However, the mean ratio of TH-/ChAT- positive nerve structures was significantly greater in samples taken from AF patients as compared to SR patients (14.26 ± 9.95 vs. 4.2 ± 1.62 , respectively, $p=0.01$). We also used ANOVA to compare TH-immunostained sympathetic nerve density

with the other nerve markers GAP43, ChAT, and SYN in AF patients. As opposed to SR patients, AF patients demonstrated a higher density of sympathetic structures than the other nerve elements ($p=0.03$). However, there was no predilection for either PV-LA junction or RAA sites ($p=ns$, Table 3).

Discussion

A complex histopathological substrate characterized by HCN4-/PAS-positive cardiomyocytes, and ICLC scattered in fibrotic tissue, inflammatory infiltrate, and sympathetic nerve structures is present in the atria and the PV muscle sleeves of chronic AF patients.

Isolated PAS-Positive Cells and Interstitial Cajal-like Cells in the PVs and Atria in Chronic AF

PAS-positive cells were reported being clustered along the endocardium of the PV muscle sleeves (8). PAS stain is used to identify glycogen-rich cells. However, glycogen rapidly degrades during ischemia, and reliable PAS-staining can only be performed on tissues fixed immediately after removal from a living heart (9). Taking live tissues from hearts instead of cadavers, allowed us to avoid glycogen degradation, and to reliably identify PAS-positive cells in the tissues.

The function of PAS-positive cells is unclear. Specialized cells have been previously identified (10). However, cellular glycogen content has not been proven to represent an evidence of “specialization”. Currently, we morphologically distinguish cardiac conduction tissues on the basis of three histological criteria proposed by Mönckeberg and Aschoff in 1910: the cells comprising the proposed tracts should be histologically distinct from their neighbors; it should be possible to follow them through serial sections; and they should be separated from the remainder non-specialized adjacent working myocardium by insulating sheaths of fibrous tissue (11,12). Nodal and transitional cells satisfy two of these criteria (13). PAS-positive myocytes of AF patients, being histologically distinct, satisfy only one criterion. These light microscopy criteria remain the gold standard for recognition of specialized myocardium, but they could be supplemented by newer techniques, including immunohistochemistry. We used HCN4 stain as an indicator of the potential electrophysiological function of PAS-positive cardiomyocytes, but we are unable to conclude that they are in fact specialized, since HCN4 expression can also be detected in non-pacemaking tissues at a lower level than in tissues with pacemaker function, and to date, pacemaking function or automaticity can only be documented by recordings of generated regular spontaneous electrical activity. Despite reports describing a close spatial relationship between PAS-positive cells and sympathetic nerves near the sites of PV muscle sleeve ectopy in canine models of AF (4,8), whether PAS-positive cardiomyocytes have pacemaker current or are a potential source of automaticity remains unknown.

The present study documents for the first time, that in chronic AF patients, abundant collagen fibers, inflammatory infiltrates and sympathetic nerve twigs surround individual PAS-positive cardiomyocytes, thus breaking up their clusters typically seen in SR patients. While we cannot conclude that they are arrhythmogenic, the new non-clustered arrangement of PAS-/HCN4-positive cardiomyocytes might have altered their electrophysiological properties. Loss of intercellular interactions such as lateral inhibition from neighboring PAS-positive cardiomyocytes, may favor automaticity, localized microreentry and AF perpetuation at the PV-LA junction.

Using autopsy tissues, Morel et al (6) recently reported the presence of interstitial Cajal cells in human PVs. However, because autopsy tissues were used, it remained unclear whether these Cajal cells were also PAS-positive. We were able to differentiate the ICLC and PAS-positive cardiomyocytes based on their morphology and distribution. The ICLC were located among

myocardial and nerve bundles, and capillaries in the atria and PV-LA junctions of AF patients, and were not the same type of cells as the PAS-positive cardiomyocytes.

Although PAS-positive cardiomyocytes, and the ICLC are not part of the specialized conduction system, they both represent sites of potential pacemaking activity within the atria and PV-LA junctions of AF patients.

Inflammatory Infiltrate and Fibrosis in the PVs and Atria in Chronic AF

Our AF patients were older and had larger atria than SR patients. They demonstrated increased fibrosis and inflammatory infiltrates in the atria and PV-LA junctions. The same findings are reported in patients with lone paroxysmal AF (14), indicating a common underlying pathogenesis. Increased myocardial inflammation and fibrosis are important factors in the generation and maintenance of AF (1,15). Fibrosis affects cellular connectivity and enhances the spatial discordance of membrane ion current handling, thus promoting triggered activity (16–18). Fibrosis also predisposes to localized microreentry of multiple AF wavelets especially at the PV sleeves where there is a complex architectural arrangement of myocardial bundles (7,19,20). However it is not clear, whether the presence of fibrosis and inflammation is a cause or effect of AF or simply an epiphenomenon representing an older patient population that is more prone to AF.

Sympathetic Neural Elements in the PVs and Atria in Chronic AF

Canine models of pacing-induced AF have demonstrated sympathetic nerve sprouting in the sites of stimulation and in their tributary innervation territories (21–23). Heterogeneous atrial sympathetic hyperinnervation is present in patients with persistent AF (3). In our study, sympathetic innervation was increased in chronic AF without reaching statistical significance. This could be due to the small sample size, a small specimen size with undersampling of patchy sympathetic innervation or to variable sensitivity of tissues to different antibodies. Animal studies demonstrated the important role of the autonomic nervous system in AF initiation and maintenance. In fact, sympathovagal imbalance causes anisotropic changes in the action potential durations and refractory periods of myocytes at the PVs sleeves and atria (24). While targeting autonomic cardiac ganglia alone did not prevent long-term AF recurrences (25), it has been shown to improve the cure rate, when used as an adjunct to surgical PV isolation (26).

Clinical Implications for Substrate Modification

Multiple underlying histopathological substrates might explain localized triggered activity and microreentrant circuits in the atria and PV-LA junctions as fundamental mechanisms for the genesis and maintenance of chronic AF in diseased hearts (19,27). Current percutaneous ablation strategies attempt to use the smallest number of lesions usually limited only to the LA. The diffuse biatrial disease found in our study might explain the lack of AF termination after the completion of the lesions sets in the LA (28). This may also be why biatrial lesions performed during the surgical Maze procedure have shown promising results (29) as compared to the left-sided lesions only (30).

These histological substrates might represent the source of the commonly observed complex atrial electrograms in the PV-LA junctions and atria. If that be true, additional electrogram-based and substrate-based lesion sets besides the achievement of PV electrical isolation should be considered in selected cases in order to terminate AF (31). The correlation of these histopathological substrates with their electrical properties is currently being investigated.

Study Limitations

The biggest limitation of this study is its small size. However, the relevance of these findings is enhanced by the fact that this was conducted on live human tissues rather than animal models mimicking AF or cadavers. Moreover, the investigators performing the histopathological examinations were blinded to the source of the tissue. It provides a realistic demonstration of the complex substrate present in the atria and PV-LA junctions. The older age and larger LA size of the AF group limit the study. These two variables can increase the presence of fibrosis. Finally, this is a purely anatomical study. Ultrastructural and electrophysiological investigations are required to define the PAS-positive cardiomyocytes and their pathophysiological significance.

Conclusions

The definition of chronic AF as a functional electrical disorder does not reflect the significant underlying structural abnormalities. Diffuse atrial and PV muscle sleeve microstructural remodeling is present, and establishes a vulnerable substrate for AF maintenance. This study demonstrates subendocardial PAS-positive cardiomyocytes that might have a potential pacemaking function, scattered among abundant inflammatory infiltrate, fibrous tissue, and sympathetic nerve structures in the atria and PV-LA junctions of patients with chronic AF. Their morphology and distribution is different from Cajal cells, and their electrophysiological significance remains to be investigated. The question led by our observations on the critical mechanistic contribution of atrial remodeling to the complex pathogenesis of chronic AF remains to be answered.

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Abbreviations and Acronyms

AF	atrial fibrillation
PV-LA	pulmonary vein-left atrium
RAA	right atrial appendage
PAS	Periodic acid-Schiff
TH	tyrosine hydroxylase

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Figure 1.
Location of the biopsies performed at the right PV-LA junctions and RAA (red crosses, Panel A). AO: aorta; CS: coronary sinus; IVC: inferior vena cava; LA: left atrium; LI: left inferior pulmonary vein; LS: left superior pulmonary vein; PA: pulmonary artery; RI: right inferior pulmonary vein; RS: right superior pulmonary vein; SVC: superior vena cava; VOM: vein of Marshall.

Figure 2.

Non-clustered PAS-positive cells at the PV muscle sleeve before (black arrows, Panel A), and after (Panel B) diastase glycogen digestion in serial tissue sections. The scattered PAS-positive cells (black arrows, Panels C and E) show anti-myoglobin positivity (black arrows, Panel D), and HCN4 positivity (black arrows, Panel F). CD117/c-kit immunostaining shows interstitial Cajal-like cells (ICLC) (arrowhead, Panel G) among myocytes, blood capillaries, and nerve bundles. The dashed arrow marks a thin cellular process (Panel H).

Figure 3. Normal pattern of distribution of clustered subendocardial PAS-positive cells (black arrows, Panel A) with minimal interstitial collagen fibers between the previously identified PAS-positive cells (Panel B) at the PV muscle sleeve in a SR patient. Pathological pattern of distribution of subendocardial PAS-positive cells (black arrows, Panel C) scattered among abundant inflammatory infiltrate and interstitial connective tissue (Panel D) at the PV muscle sleeve in a patient with chronic AF.

Figure 4.

Individual PAS-positive cells (black arrows, Panels A and D), surrounded by abundant collagen fibers, fibroblasts, inflammatory infiltrate (Panels B and E), and sympathetic nerve twigs (black arrows, Panels C and F) in chronic AF. A, B, and C are taken from the right atrial appendage; D, E, and F are taken from the PV muscle sleeve. Panel G shows the comparison of intramyocardial sympathetic nerve structures in chronic AF and SR patients and other nerve structures at the PV muscle sleeve, and right atrial appendage.

Figure 5. Coexistence of clusters of chronic lymphomononuclear inflammatory infiltrate (black arrows, Panel A) associated with pronounced vacuolar degeneration of the adjacent atrial myocytes, and extensive patchy interstitial replacement with collagen fibers separating atrial myocytes (black arrows, Panel B) from the neighboring ones at the PV muscle sleeve in chronic AF.

Table 1
Clinical characteristics of the 8 patients enrolled in the study

	AF N=5	SR N=3	P
Age, years±SD	77.4±10.5	60±2.6	0.03
Female, %	80 (4/5)	66.7 (2/3)	1
Caucasian, %	80 (4/5)	100 (3/3)	1
MVD, %			
MS	20 (1/5)	0 (0/3)	1
MR	80 (4/5)	100 (3/3)	0.2
Etiology, %			
Degenerative	20 (1/5)	0 (0/3)	1
Rheumatic	40 (2/5)	0 (0/3)	0.46
Dilated cardiomyopathy	60 (3/5)	66.7 (2/3)	1
Myxomatous	0 (0/5)	66.7 (2/3)	0.11
Ehlers-Danlos Syndrome	20 (1/5)	0 (0/3)	1
Duration of AF, months±SD	58±51.33	0	n/a
Hypertension, %	80 (4/5)	100 (3/3)	1
CAD, %	0 (0/5)	0 (0/3)	1
Diabetes mellitus, %	0 (0/5)	0 (0/3)	1
NYHA III-IV, %	100 (5/5)	66.7 (2/3)	0.38
LVEF, mean±SD	0.45±0.20	0.62±0.08	0.2
LAEDD, mm±SD	55.6±4.6	44±3	0.009
LVEDD, mm±SD	58.8±6.4	53±10.5	0.4
Drugs, %			
Beta-blockers	100 (5/5)	100 (3/3)	1
ACE-inhibitors	100 (5/5)	100 (3/3)	1
Digoxin	20 (1/5)	0 (0/3)	1

ACE: angiotensin converter enzyme; AF: atrial fibrillation; LAEDD: left atrial end-diastolic diameter; LVEDD: left ventricular end-diastolic diameter; LVEF: left ventricular ejection fraction; MR: mitral regurgitation; MS: mitral stenosis; MVD: mitral valve disease; NYHA: New York Heart Association; n/a: not applicable; SR: sinus rhythm.

Table 2
Histological and immunohistochemical results in AF and SR patients

	AF n=5	SR n=3	P
Inflammatory infiltrates, %	100 (5/5)	33.3 (1/3)	0.002
Fibrosis, %±SD	37±5.6	7.4±2.8	0.009
THc +, $\mu\text{m}^2/\text{mm}^2$	5009.8±1544.6	1781.8±275.8	0.17
GAP43c +, $\mu\text{m}^2/\text{mm}^2$	2503.4±723.1	2521.3±1061.6	1
SYNc +, $\mu\text{m}^2/\text{mm}^2$	2523.8±641.4	3269.6±1272.2	0.6
ChATc +, $\mu\text{m}^2/\text{mm}^2$	351.4±155.2	424.3±170.3	0.8
PAS + cell layer thickness, mm±SD	0.07±0.006	0.08±0.02	0.29
Presence of non-clustered PAS + cells, %	80 (4/5)	0 (0/3)	0.04
Size of:			
Clustered PAS + cells, mm±SD	0.04±0.002	0.032±0.002	0.08
Non-clustered PAS+ cells, mm±SD	0.04±0.002	n/a	n/a
PAS - cells, mm±SD	0.02±0.001	0.02±0.001	0.18
Presence of ICLC, %	100 (5/5)	100 (5/5)	1

AF: atrial fibrillation; ChATc: corrected choline acetyltransferase; GAP43c: corrected growth-associated protein 43; ICLC: interstitial Cajal-like cells; n/a: not applicable; PAS: Periodic-Acid Schiff; SR: sinus rhythm; SYNc: corrected synaptophysin; THc: corrected tyrosine hydroxylase; +: positive; -: negative.

Comparison of the histological and immunohistochemical findings between PV-LA junction and RAA samples of chronic AF and SR patients

Table 3

	AF PV-LA n=10	AF RAA n=5	SR PV-LA n=6	SR RAA n=2	P
Inflammatory infiltrate, %	100 (10/10)	100 (5/5)	33.3 (2/6)	50 (1/2)	0.0082 [*]
Fibrosis, %±SD	38.8±24.7	33.2±15.6	5.5±5.6	13±14.1	0.006 [*] and 0.003 [†]
THc +, μm ² /mm ²	3523.9±4371.9	7981.5±8104.9	1662.6±2589	2139.3±467.6	ns
Presence of non-clustered PAS + cells, %	70 (7/10)	80 (4/5)	0	0	0.01 [*]

^{*} for PV-LA junction in AF vs. SR;

[†] for RAA in AF vs. PV-LA junction in SR

AF: atrial fibrillation; ns: not significant; PAS: Periodic-Acid Schiff; PV-LA: pulmonary vein-left atrium; RAA: right atrial appendage; SR: sinus rhythm; THc: corrected tyrosine hydroxylase; +: positive.