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Role of tissue C-reactive protein in atrial cardiomyocytes of patients undergoing catheter ablation of atrial fibrillation: pathogenetic implications

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Aims Histological studies support the important role of inflammation in the initiation and maintenance of atrial fibrillation (AF). We describe a novel and safe technique of atrial biopsy during AF radiofrequency catheter ablation (RFCA) to investigate the role of atrial tissue inflammation.

Methods and results We enrolled 70 consecutive patients (age 60 ± 12 years, 49 males) undergoing RFCA for AF. The control group was represented by 10 patients with Wolff–Parkinson–White syndrome undergoing trans-septal puncture. Atrial biopsies were obtained by washing the dilator and needle used for trans-septal puncture with 20 mL sterile phosphate-buffered saline. The presence of intracytoplasmic C-reactive protein was assessed in formalin-fixed atrial specimens by immunohistochemistry. A sufficient amount of atrial tissue was obtained in 23/70 (32%) patients with AF and in 4/10 (40%) of the control group. Intracytoplasmic localization of C-reactive protein was found in isolated atrial cardiomyocytes in 11 (73%) of 15 patients with paroxysmal AF as compared with 2 (25%) of eight patients with persistent AF ($P = 0.02$).

Conclusion In this study, we demonstrate the safety and feasibility of a novel technique to obtain atrial specimens during routine trans-septal puncture. Local inflammation assessed by atrial tissue localization of C-reactive protein is more likely involved in paroxysmal rather than in persistent AF.

Keywords Atrial fibrillation • Trans-septal puncture • Atrial endomyocardial biopsy • C-reactive protein

Introduction

Atrial fibrillation (AF) is a growing public health burden because of its increasing incidence, its potential for devastating clinical consequences, and its complex and expensive treatment.[1,2] Thus, new prevention strategies are highly desirable. Histological and biomarker studies support the notion of a robust link between inflammatory status and AF.[3] In particular, atrial specimens obtained by endomyocardial biopsy (EMB) showed clusters of lymphomononuclear cells compatible with diagnosis of myocarditis in 66% of patients with lone AF.[4] Furthermore, in patients with non-valvular AF undergoing surgical thrombectomy with left atrial appendectomy because of large thrombi, activated T lymphocytes were found in left atrial endocardium, supporting the role of local inflammation as a potential trigger of AF.[5] Thus, atrial EMB may represent a valid tool to elucidate cellular and molecular mechanisms of inflammation associated with AF. This procedure, however, is currently indicated only in patients with atrial cardiac

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tumours,[6–9] while current guidelines recommend that it should not be performed in the setting of unexplained AF,[10] as the risks are not counterbalanced by a clear clinical benefit. The safety of trans-septal catheterization in the setting of radiofrequency catheter ablation (RFCA) has been established by several studies.[11–13] Notably, its success rate when guided by intracardiac echography has been reported to reach 100%.[14–16] Thus, as trans-septal catheterization is routinely used for RFCA of AF, we have developed a novel approach for assessing the histopathology of AF using atrial specimens obtained during routine trans-septal catheterization.

Methods

Patient characteristics

We prospectively enrolled 70 consecutive patients (mean age was 60 ± 12 years, 49 males) with paroxysmal or persistent AF undergoing antral pulmonary vein isolation. Atrial fibrillation was paroxysmal in 42 patients and persistent in 28 patients, according to the definition given by the recent European Society of Cardiology guidelines.[17] Exclusion criteria included intercurrent infective and inflammatory disorders, neoplastic disease, treatment with steroid or non-steroidal anti-inflammatory agents other than aspirin (up to 100 mg daily), an acute coronary syndrome within the month before catheter ablation and patent foramen ovale. We also enrolled 10 control patients (mean age 23 ± 7 years, 3 females) with Wolff–Parkinson–White (WPW) syndrome who underwent catheter ablation of left-sided accessory pathways, without history of AF at the time of ablation. The protocol was approved by the Ethic Committee of the Catholic University of Sacred Heart and all patients provided written informed consent.

Electrophysiological procedure

Before the procedure, computerized tomography scan of the heart was performed in all patients to define the anatomy of the pulmonary veins (PVs) and transthoracic echocardiography to assess left atrial parameters. In all patients trans-septal catheterization was performed by trans-septal assembly consisting of (i) 8F Preface sheath (Biosense Webster, Diamond Bar, CA, USA), (ii) dilator, and (iii) Brockenbrough needle. All patients underwent trans-septal puncture and AF ablation using an intracardiac echocardiography-guided technique.[18,19] We also used dye injection to confirm the correct positioning of the needle in the left atrium, as soon as the most distal part of the dilator had passed the atrial septum at the fossa ovalis. Intracardiac echocardiography-guided PVs antrum isolation was performed under the guidance of Carto mapping system (Biosense Webster) in 65% of patients and EnSite NavX mapping system (St Jude Medical, Inc., St Paul, MN, USA) in 35% of patients. Irrigated radiofrequency energy was delivered with a target temperature of 43°C and a power between 25 and 35 W (Thermo Cool, Biosense Webster). The electrophysiological endpoint was absence or dissociation of all PVs potentials documented by circular mapping catheter (Lasso, Biosense Webster) within the ipsilateral superior and inferior PVs and along the PVs antrum.[20] No additional lines were made in paroxysmal and persistent AF patients.

Atrial specimens sampling

After single trans-septal puncture and advancement of trans-septal assembly in the left atrium, the dilator and the needle were removed and separately flushed with 10 mL of phosphate buffer solution (PBS 10× GIBCO, Invitrogen) in 20 mL tube. Trans-septal puncture was performed in the septal region of the right atrium, from areas adjacent to the fossa ovalis, as indicated by intracardiac echocardiography. After this step, all patients were systematically anticoagulated with intravenous heparin to reach an activated clotting time of 300–400 s. After removal of PBS the atrial specimens were immersed in 10 cc of 10% formalin.

Immunohistochemistry

The formalin-fixed, paraffin-embedded specimens were sectioned at 5 μm and stained with haematoxylin and eosin and with haematoxylin and Van Gieson to confirm the presence of atrial cardiomyocytes. Sections from each specimen displaying atrial cardiomyocytes were cut at 5 μm, mounted on glass, and dried overnight at 37°C. All sections were then deparaffinized in xylene, rehydrated through a graded alcohol series and washed in PBS, which was used also for all subsequent washes and for antibody dilution. Endogenous peroxidase activity was blocked by 5% hydrogen peroxide. For immunohistochemistry, tissue sections were heated twice in a microwave oven for 5 min each at 700 W in citrate buffer (pH 6). The murine monoclonal antibody (clone C-reactive protein-8, IgG1, Sigma, St Louis, MO, USA) directed against human C-reactive protein was used at 1:100 dilution and incubated overnight at 4°C. Then, the sections were processed with the standard streptavidin-biotin-immunoperoxidase method (DAKO Universal Kit, DAKO Corp., Carpinteria, CA, USA). Diaminobenzidine was used as the final chromogen, and haematoxylin as the nuclear counterstain. The monoclonal antibody C-reactive protein-8 displayed its reactivity against native and denatured C-reactive protein without cross-reaction with human serum, amyloid P component, human haptoglobin, human alpha-1-acid glyco-protein, and human IgG. All specimens were analysed to assess cell number and the immunoreactivity to C-reactive protein by a single investigator who was blinded to patients' characteristics. Atrial specimens were scored as positive or negative for the presence or absence of C-reactive protein.

Serum high-sensitivity C-reactive protein assay

Serum high-sensitivity-C-reactive protein was assayed at baseline, immediately after pulmonary vein isolation, and on Days 1 and 2 post-operatively using a latex-enhanced immunonephelometric assay (BN II, Siemens Diagnostic, Glasgow, DE, USA) as described elsewhere.[21]

Follow-up

After the procedure, patients continued anticoagulation with warfarin to maintain an international normalized ratio of 2.0–3.0 for a minimum of 3 months. In all patients, antiarrhythmic medications were continued for 2 months after RFCA and they were chosen from one of sotalol, propafenone, and flecainide. Amiodarone was not used after ablation. Antiarrhythmic medications were discontinued in all patients after 2 months.

Early recurrence or late recurrence of AF was defined as AF occurring within or beyond 2 months of RFCA, respectively.[22] Patients had scheduled clinical visits, 12-lead electrocardiography (ECG), and 24 h Holter monitoring at 1, 2, 3, 6, and 12 months after catheter ablation. Patients were also advised to monthly report any recurrence of symptoms; in this case, 24 h Holter monitoring was promptly obtained. Recurrences were based on patient reporting, Holter, and ECG data. The mean follow-up time for all patients in this study was 17 ± 8 months.

Statistical analysis

Continuous variables are reported as the mean value ± standard deviation (SD) or the median value and range for skewed distributions, whereas categorical variables as absolute frequencies and percentages. Comparisons between groups were performed with the unpaired Student *t* test, the Mann–Whitney *U* test, or χ^2 test as appropriate. All tests were two sided, and a value of $P < 0.05$ was settled for statistical significance. Statistical analyses were performed by STATA 10.0 (Stata Corporation, College Station, TX, USA) statistical package.

Results

Clinical findings

Baseline characteristics of patients with paroxysmal or persistent AF with adequate atrial samples are shown in *Table 1*. No significant differences were observed between the two groups with regard to age, gender, or prevalence of hypertension, coronary artery disease, or use of statin, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers, beta-blockers, amiodarone, IC antiarrhythmic agents. Early recurrence of AF rates was 26% among patients with paroxysmal AF and 38% among patients with persistent AF. After a mean follow-up of 17 ± 8 months, late recurrence of AF rates were 40% among patients with paroxysmal AF and 13% among patients with persistent AF.

Histological findings and clinical correlates

A total of 23 out of 70 atrial specimens from patients with AF were adequate for pathological evaluation for a 32% yield (*Figure 1A* and *B*). The presence of atrial cardiomyocytes was confirmed by histological analysis performed by a blinded expert pathologist. Of note, atrial specimens could not be obtained from any of the five patients in whom trans-septal puncture was performed with atrial radiofrequency current delivery to cross-resistant interatrial septum.[23] Atrial specimens from 4 (40%) out of 10 patients with left-sided accessory pathways were adequate for immunostaining.

The number of isolated atrial cardiomyocytes per slide ranged from a minimum of 60 to a maximum of 120 cells. More than 90% of isolated atrial cardiomyocytes retained cellular and nuclear integrity. Atrial cardiomyocytes appeared normal in size and structure in all patients with AF and in the control group (*Figure 1C–F*). Immunohistochemical analysis revealed C-reactive protein staining in 13 out of the 23 atrial specimens from patients with AF, with specific localization of C-reactive protein within the cytoplasm of atrial cardiomyocytes

Table 1 Baseline characteristics of patients with paroxysmal and persistent atrial fibrillation

	Paroxysmal AF (*n* 15)	Persistent AF (*n* 8)	*P*
Male gender, *n* (%)	10 (67)	6 (75)	1
Age	61 ± 11	59 ± 9	0.65
AF duration (years)	5.6 ± 3.5	6.6 ± 6	0.67
Lone AF, *n* (%)	8 (53)	6 (74)	0.39
Structural heart disease			
Hypertensive cardiomiopathy, *n* (%)	6 (40)	1 (13)	0.35
Hypertrophic cardiomiopathy, *n* (%)	1 (7)	0 (0)	1
Valvular heart disease, *n* (%)	0 (0)	1 (13)	0.35
Echocardiographic parameters			
Left atrial diameter (mm)	43.8 ± 7	44.8 ± 7.9	0.77
Left atrial volume (mL)	91.1 ± 23.2	98.8 ± 32.8	0.56
LVEF (%)	60.2 ± 7.2	53.9 ± 8.2	0.08
CT parameters			
Left atrial volume (mL)	154.5 ± 103.6	161.4 ± 69.5	0.85
Procedure time (min)	271.2 ± 58.1	282.9 ± 51.9	0.58
Pre-ablation medication			
Beta-blockers, *n* (%)	3 (20)	5 (63)	0.07
Amiodarone, *n* (%)	4 (27)	4 (50)	0.37
IC antiarrhythmic agents, *n* (%)	6 (40)	6 (63)	0.19
Statin, *n* (%)	3 (20)	1 (13)	0.62
ACEIs/ARB, *n* (%)	9 (60)	4 (57)	0.68
Early atrial fibrillation recurrence (ERAF), *n* (%)	4 (26)	3 (38)	0.66
Late atrial fibrillation recurrence (LRAF), *n* (%)	6 (40)	1 (13)	0.34
Follow-up (months)	10 ± 6	14 ± 8	0.23

Values are presented as mean ± SD, or *n* (%).
AF, atrial fibrillation; LVEF, left ventricular ejection fraction; CT, computed tomography; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockers.

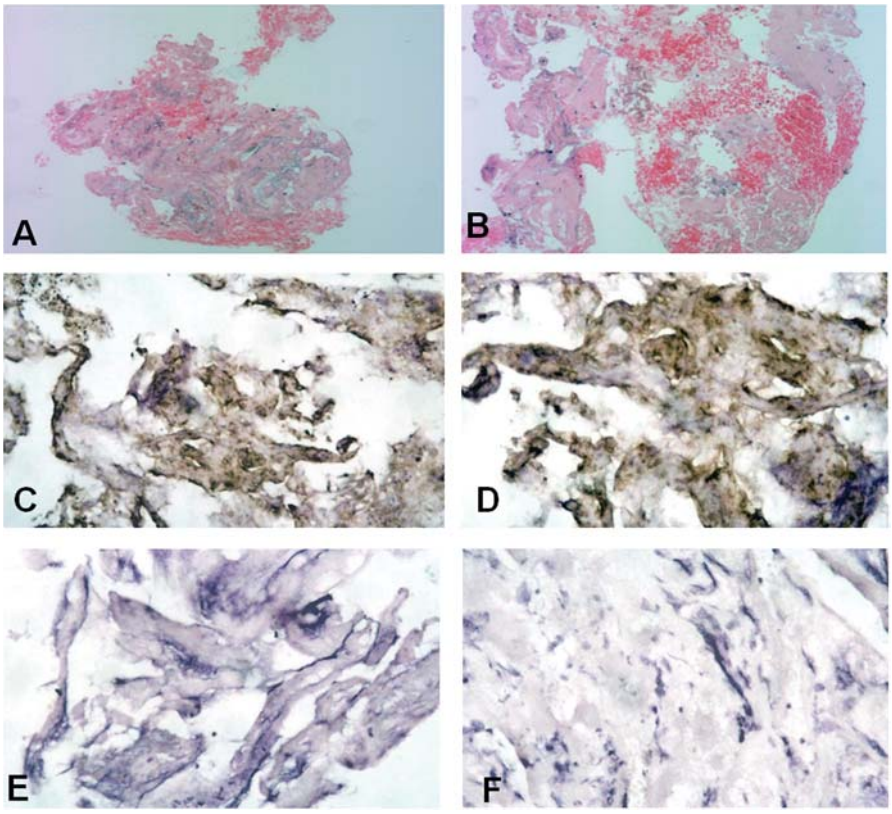

Figure 1 Atrial cardiomyocytes isolated by trans-septal assembly washing. (*A*, *B*) Serial sections of isolated atrial cardiomyocytes were stained with haematoxylin/eosin (original magnification 4×); (*C*, *D*) Atrial cardiomyocytes of patients with atrial fibrillation were immunohistochemically stained with C-reactive protein antibodies. Positive staining for C-reactive protein is noted as brown in the cytoplasm with diffuse pattern of localization (original magnification 20×); (*E*, *F*) negative staining for C-reactive protein was found in atrial cardiomyocytes of patients with left-sided accessory pathways (original magnification 20×).

(*Figure 1C* and *D*). C-reactive protein immunostaining was absent in all four atrial specimens of patients with left-sided accessory pathways (*Figure 1E* and *F*).

The characteristics of patients according to the presence or absence of C-reactive protein in atrial specimens are depicted in *Table 2*. Mean age, presence of AF at the time of pulmonary vein isolation, incidence of structural heart disease, echocardiographic, and CT left atrial parameters were similar between patient positive or negative for atrial tissue C-reactive protein. The percentage of patients taking statins and angiotensin-converting enzyme inhibitors also did not differ between atrial tissue C-reactive protein-positive and C-reactive protein-negative groups. Conversely, a higher proportion of patients positive for atrial tissue C-reactive protein presented paroxysmal AF as compared with those negative for tissue C-reactive protein (85 vs. 40%, $P = 0.02$) (*Figure 2*). Also, the rate of late recurrence of AF was higher in patients positive for atrial tissue C-reactive protein as compared with those negative for atrial tissue C-reactive protein, although this difference achieved borderline significance (46 vs. 10%, $P = 0.06$), while the rate of early recurrence of AF was similar in patients positive or negative for tissue C-reactive protein (*Table 2*).

Serum high-sensitivity C-reactive protein levels

Among patients with AF, high-sensitivity-C-reactive protein levels significantly increased from baseline to 24 and 48 h post-operatively (baseline: median 1.725 mg/L, range 0.08–94 mg/L; immediately post-RFCA: median 1.9 mg/L, range 0.08–12 mg/L; 24 h after RFCA: median 15.6 mg/L, range 7.84–211 mg/L; 48 h after RFCA: median 39.3 mg/L, range 16.6–183 mg/L; all $P < 0.001$) (*Figure 3*). At each time point, no difference was observed between patients positive or negative for tissue C-reactive protein (*Figure 4A*), with paroxysmal or persistent AF (*Figure 4B*), with or without late recurrence of AF, with or without early recurrence of AF.

Discussion

This study demonstrates the feasibility of harvesting atrial cardiomyocytes by trans-septal catheterization. Atrial EMB in patients with AF should be avoided, as recommended by a recent AHA/ACC/ESC scientific statement because of safety concerns.[10] In

Table 2 Demographics and instrumental findings of patients with and without atrial tissue C-reactive protein

	Atrial tissue C-reactive protein Negative (*n* 10)	Atrial tissue C-reactive protein Positive (*n*13)	*P*
Male gender, *n* (%)	6 (60)	10 (77)	0.4
Age (years)	64 ± 5	57 ± 12	0.08
Paroxysmal atrial fibrillation, *n* (%)	4 (40)	11 (85)	0.009*
Persistent atrial fibrillation, *n* (%)	6 (60)	2 (15)	0.009*
Atrial fibrillation at the time of catheter ablation, *n* (%)	5 (50)	8 (62)	0.6
Lone atrial fibrillation, *n* (%)	7 (70)	7 (54)	0.5
Structural heart disease, *n* (%)	3 (30)	6 (46)	0.5
Echocardiographic parameters			
Left atrial diameter (mm)	47 ± 2	42 ± 8	0.3
Left atrial volume (mL)	107 ± 22	85 ± 25	0.1
LVEF (%)	57 ± 9	58 ± 7	1
CT Left atrial volume (mL)	155 ± 29	160 ± 106	0.4
hs C-reactive protein serum levels (mg/dL)			
Pre-ablation	2.1 (1.2–2.5)	1.3 (0.8–6.8)	0.8
Post-ablation	2.4 (1.8–3.2)	1.6 (0.8–1.9)	0.07
24 h after ablation	15.2 (10.7–18.2)	18 (13.4–20.2)	0.4
48 h after ablation	39.7 (28.2–40.9)	38 (28.9–41)	0.7
Early atrial fibrillation recurrence (ERAF), *n* (%)	3 (30)	4 (30)	0.9
Late atrial fibrillation recurrence (LRAF), *n* (%)	1 (10)	6 (46)	0.06

Values are presented as mean ± SD, median (interquartile range), or *n* (%). LVEF, left ventricular ejection fraction; CT, computed tomography; hs, high sensitivity.
*Denotes statistical significance.

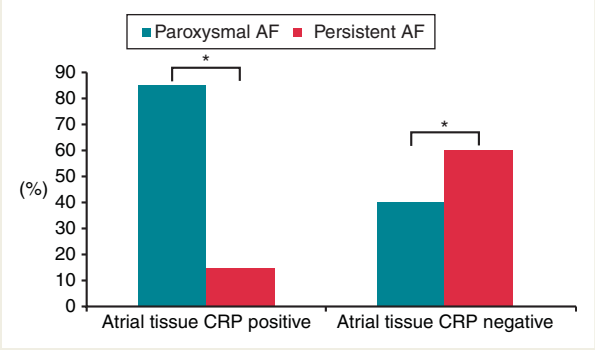

Figure 2 Intracytoplasmic C-reactive protein (CRP) and diagnosis of atrial fibrillation. Atrial specimens (%) positive and negative for tissue C-reactive protein in patients with diagnosis of paroxysmal and persistent atrial fibrillation (AF). Asterisk indicates all $P = 0.02$.

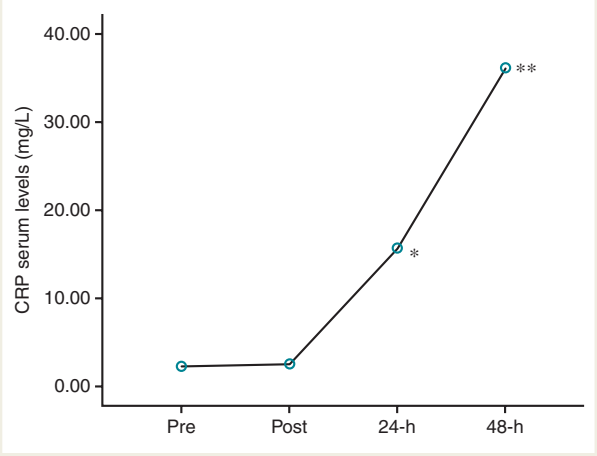

Figure 3 Temporal trend of serum high-sensitivity C-reactive protein (CRP) in all patients with atrial fibrillation. High-sensitivity C-reactive protein was assayed at baseline (pre), immediately after pulmonary vein isolation (post), and at 24 h and 48 h after radiofrequency catheter ablation. Asterisk indicates $P < 0.001$ vs. pre-operative C-reactive protein assay (pre), Double asterisk indicates $P < 0.001$ vs. pre and vs. 24 h.

contrast to the risks associated with this procedure, trans-septal left heart catheterization is a safe technique and its clinical use is expanding. Early experience with small numbers of patients demonstrated the safety of performing trans-septal catheterization for ablation of accessory pathways and atrial tachycardias.[11] In recent series, including over 5000 patients most of whom underwent ablation of AF, complication rates associated with trans-septal access were <1%.[12] With the advent of intracardiac echocardiography, the success rate in trans-septal catheterization has been reported to reach 100%; thus, this technique is routinely utilized in patients undergoing catheter ablation of AF or of left-sided accessory

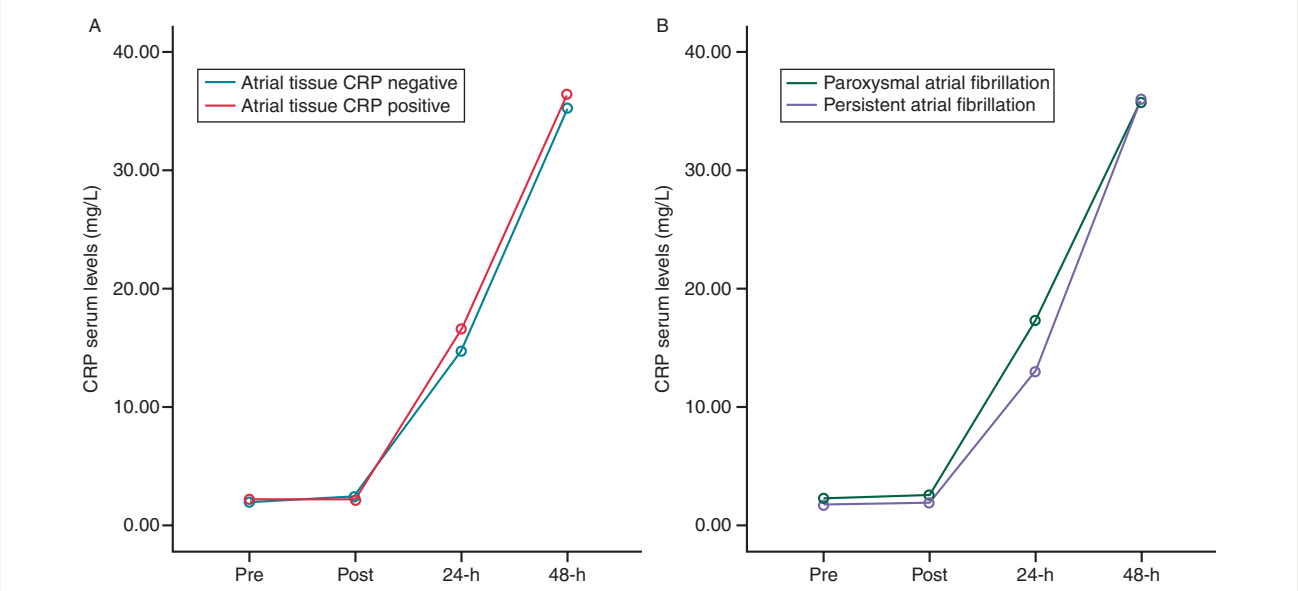

Figure 4 (*A*) High-sensitivity C-reactive protein (CRP) was assayed at baseline (pre), immediately after pulmonary vein isolation (post), and at 24 h and 48 h after radiofrequency catheter ablation in patients positive and negative for intracytoplasmic C-reactive protein ($P = 0.7$). (*B*) High-sensitivity C-reactive protein was assayed at baseline (pre), immediately after pulmonary vein isolation (post), and at 24 h and 48 h after radiofrequency catheter ablation in patients with diagnosis of paroxysmal and persistent atrial fibrillation ($P = 0.2$).

pathway.[14–16] Accordingly, no complication associated with trans-septal catheterization was observed in our study.

An atrial tissue specimen as by-product of routine trans-septal puncture was suitable for immunohistochemistry only in 32 and in 40% of patients with AF and with left-sided accessory pathways, respectively. Interestingly, positivity for C-reactive protein in atrial cardiomyocytes was not found in any of the four atrial specimens from patients with left accessory pathways, whereas atrial tissue C-reactive protein was significantly more frequent in patients with paroxysmal AF than in patients with persistent AF. Furthermore, we observed a trend for an association between positivity for atrial tissue C-reactive protein and late recurrence of AF.

An increasing body of evidence links atrial inflammation to at least some types of AF, as non-valvular AF,[5] lone paroxysmal AF[4], and AF associated with pericarditis.[3,24] Among patients with lone paroxysmal AF, results of atrial EMB demonstrated cardiomyopathic alterations, such as atrial myocarditis, patchy fibrosis, myocyte hypertrophy, and vacuolar degeneration of atrial myocytes due to an extensive myofibrillolysis.[4] In particular, Frustaci *et al.*[4] found atrial inflammatory lymphomononuclear infiltrates in 66% of patients with lone AF and normal histology in 11 atrial specimens from WPW patients. In this study, atrial EMB was performed in the septal region of the right atrium, from areas adjacent to fossa ovalis. Our atrial specimens were also obtained from fossa ovalis. Atrial musculature in the fossa ovalis consists of muscular bands isolated by fatty tissue from the endocardium of the right and left atrium.[25]

Our population also was predominantly composed of lone AF patients (53% of patients with paroxysmal AF and 74% of patients with persistent AF). The higher proportion of atrial C-reactive protein-positive specimens in paroxysmal AF than in persistent AF group may be an initial feature of myocardial damage via activation of the complement system, opsonization, chemotaxis, and activation of inflammatory cells by this acute phase protein.[26,27] A recent study by cardiac magnetic resonance demonstrated atrial delayed enhancement, a marker of fibrosis more frequently in persistent AF as compared with paroxysmal AF. Thus, in paroxysmal AF the role of other mechanisms as cellular dysfunction driven by inflammation may precede the development of fibrosis.[28] In this setting of AF, atrial tissue C-reactive protein may directly contribute to these functional mechanisms acting as an opsonin and participating in the clearance of apoptotic myocyte.[29] Myocyte loss is typically accompanied by replacement fibrosis. Thus, local inflammatory response in the atrium of patients with paroxysmal AF may be the initial part of the structural remodelling, which progresses to atrial fibrosis in persistent AF.[28] Of note, C-reactive protein has been shown to localize also in ventricular cardiomyocytes after acute myocardial infarction[30–32] and in ventricular myocardium of patients with non-ischaemic dilated cardiomyopathy.[33] In ischaemic ventricular cardiomyocytes, C-reactive protein localization pattern suggested that this protein recognized membrane epitopes expressed on jeopardized myocytes thus binding to sarcolemma.[31] In contrast, in ventricular myocardium of patients with non-ischaemic dilated cardiomyopathy there was a significant expression of C-reactive protein and tumour necrosis factor alpha as proinflammatory mediators.[33] The results of our study do not allow deducing whether atrial tissue C-reactive protein is taken in or synthesized by atrial cardiomyocytes.

It is worth noting that in our study the presence of C-reactive protein in human atrial tissue was unrelated to serum high-sensitivity-C-reactive protein levels in AF patients. In particular, serum C-reactive protein levels were similar in paroxysmal and

persistent AF. In concordance with recent evidence,[34] serum high-sensitivity-C-reactive protein levels significantly increased from baseline to 48 h after catheter ablation, irrespective of the type of AF. This post-operative elevation of serum C-reactive protein is likely caused by the extensive amount of left atrial tissue involved in RFCA of AF, whereas the presence of atrial tissue C-reactive protein was assessed before RFCA.

Interestingly, Marcus *et al.*[35] by sampling from left atrium and coronary sinus demonstrated intracardiac sequestration of C-reactive protein in patients with AF as compared with patients with sinus rhythm. Consistent with these findings, we could hypothesize a systemic origin of atrial tissue C-reactive protein.

In our study, we found a borderline association between atrial tissue C-reactive protein and late recurrence of AF, but a larger sample size is needed to demonstrate the predictive role of atrial tissue C-reactive protein on late recurrence of AF. In contrast, serum high-sensitivity-C-reactive protein levels were similar in patients experiencing early and late recurrence of AF. This finding was in concordance with those reported by Letsas *et al.*[36] in patients with paroxysmal and persistent AF in which high-sensitivity-C-reactive protein was not an independent predictor of arrhythmia relapse. Lellouche *et al.*[34] demonstrated that serum C-reactive protein levels were similar in patients with and without late recurrences. Accordingly, in a large population of patients with AF, Verma *et al.*[22] found similar levels of serum C-reactive protein levels in patients with and without late arrhythmia recurrences.

The main limitation of this study is represented by the small sample size. Thus, our data should be considered as hypothesis generating. Of note, no power calculation could be performed as no previous study addressed this issue. A second limitation is sampling error enhanced by the small amount of tissue that was possible to obtain from each patient. In this regard, atrial cardiomyocytes yield from specimens obtained by trans-septal assembly washing was not compared with conventional atrial EMB. Additional technical issues could be performed in order to increase atrial cell yield. A third limitation is failure to detect asymptomatic episodes of AF during follow-up, with consequent underestimation of the incidence of recurrences.

In conclusion, this study describes a novel technique for harvesting atrial cardiomyocytes in patients with AF undergoing trans-septal catheterization for pulmonary vein isolation. The chance to obtain atrial biopsy by washing trans-septal assembly may advance our understanding of cellular and molecular mechanisms of AF initiation and progression. In our study, atrial inflammation, as assessed by the identification of C-reactive protein in isolated atrial cardiomyocytes, was more frequent in paroxysmal rather than in persistent AF patients, potentially unmasking an early functional damage of atrial cardiomyocytes leading to atrial fibrosis.

Conflict of interest: none declared.

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