

Structural Abnormalities in Atrial Walls Are Associated With Presence and Persistency of Atrial Fibrillation But Not With Age

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Objectives	The purpose of this study was to assess the association between structural changes in human atria, age, and history of atrial fibrillation (AF).
Background	Development of fibrosis in atrial walls is associated with deterioration of atrial conduction and predisposes to AF in experiment. Human data, however, are scarce, and whether fibrosis is a cause or consequence of AF is not known.
Methods	Medical records for consecutive autopsies were checked for AF history and duration. Atrial specimens from 30 patients (ages 64 ± 12 years) were collected in 3 equal age-matched groups as patients without AF history, with paroxysmal AF, or with permanent AF. Tissue samples were obtained at the level of superior pulmonary veins, inferior pulmonary veins, center of posterior left atrial wall, terminal crest, and Bachmann's bundle. Histology sections were assessed for extent of fibrosis, fatty tissues, and inflammatory infiltration at each location.
Results	No correlation was observed between age and fibrosis at any location. Fibrosis extent and fatty infiltration were twofold to threefold higher at all locations in patients with history of AF and correlated with lymphomononuclear infiltration. Patients with permanent AF had greater fibrosis extent than did patients with paroxysmal AF.
Conclusions	In post-mortem material, structural changes in the atria were not associated with age, but were significantly correlated with presence of AF and its severity. Our findings suggest that age-related changes per se are unlikely to be the sole cause of advanced fibrosis underlying AF. (J Am Coll Cardiol 2011;58:2225-32) © 2011 by the American College of Cardiology Foundation

Structural atrial remodeling is a well-described phenomenon in patients with atrial fibrillation (AF). Alterations in atrial structure and function associated with AF are commonly expressed as left atrial enlargement and reduced contractility that are likely to be caused by cardiomyocyte and interstitial changes observed in animal models of AF (1,2). Whether fibrotic transformation of atrial myocardium is a cause or consequence of AF in patients with cardiovascular disease remains a matter of debate. Aging is associated with the risk of developing AF, which has commonly been

explained by the age-dependent change in cellular electrophysiological properties shown in experiment (3) as well as loss and isolation of atrial myocardium and associated atrial conduction disturbances predisposing to AF (4).

Recent developments in magnetic resonance imaging resulted in the development of methodology aimed at detecting the presence of atrial fibrosis with delayed enhancement by using extracellular gadolinium-based contrast agents (5). Using this technique, the presence and extent of atrial fibrosis showed an association with AF persistency (6)

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and is similar in AF patients regardless of any comorbidities, thus suggesting a close pathophysiological link between atrial fibrosis and arrhythmogenesis (7). However, human histology data that would prove the validity of this concept in clinical settings are scarce. Available reports concern

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**Abbreviations
and Acronyms**

- AF** = atrial fibrillation
- BB** = Bachmann's bundle
- CT** = crista terminalis
- IPV** = inferior pulmonary vein
- LA** = left atrium
- PermAF** = permanent atrial fibrillation
- PLA** = posterior left atrial wall
- PxAF** = paroxysmal atrial fibrillation
- SPV** = superior pulmonary vein

mostly patients with mitral valve disease (8). A single report (9) on patients with lone AF reported an association between the presence of fibrosis and the history of AF. Although the association between age, atrial fibrosis extent, and development of post-operative AF has been reported in patients undergoing open-heart surgery in several studies (10–12), those investigations were based on the atrial appendage tissue samples. A systematic qualitative and quantitative study of atrial tissues from both chambers of nonvalvular AF is lacking.

In this study, we analyzed the extent of histological abnormalities in the left atrium (LA) and major atrial conduction routes to elucidate any correlation between the presence and clinical types of AF, age, and comorbidities.

Methods

Study design. After local ethics approval for medical research, 276 autopsies at the Federal Heart, Blood, and Endocrinology Centre (St. Petersburg, Russia) from January 1, 2009, to June 15, 2010, were reviewed. To minimize selection bias impact, medical records of consecutive cases of in-hospital deaths referred for post-mortem studies were screened for AF presence and clinical type. Patients with AF after open-heart surgery were excluded. We compared 3

groups of 10 subjects each, all without significant valvular disease: no indication of AF history (NoAF group); history of paroxysmal or repeatedly cardioverted persistent AF (PxAF group); and history of permanent AF with duration of at least 1 year before death (PermAF group).

Tissue sample collection and handling. Transmural atrial tissue samples of at least 20 mm × 3 mm were collected from 5 locations that included major atrial conduction pathways and posterior LA region in the vicinity of pulmonary vein ostia (Fig. 1). The sites were as follows: crista terminalis (CT) at right atrium lateral wall; Bachmann's bundle (BB) from the superior portion of the interatrial groove between the atria; posterior LA wall at superior pulmonary vein (SPV) level; centrally between pulmonary vein ostia (posterior left atrial [PLA] wall); and at inferior pulmonary veins (IPV) level.

Atrial tissue samples were fixed in 10% buffered formalin and embedded in paraffin. Sections (2 μm thick) were cut parallel to the atrial wall plane and stained with Masson's trichrome stain (Fig. 2).

Specimens were examined with computer-assisted morphometric analysis using the Leica LAS Image Analysis System (LeicaQWin Plus v3, Leica Microsystems Imaging Solutions, Cambridge, United Kingdom). The percentage of fatty infiltration, interstitial fibrosis, capillary density, and mean cardiomyocyte diameter from within each sample were assessed at ×200 magnification and calculated at 10 fields of view by a single investigator blinded to clinical and demographic data. Epicardial, endocardial, and perivascular fibrosis were excluded in assessing fibrosis percentage. A mean of the 10 measurements for each parameter per location was used for further analysis.

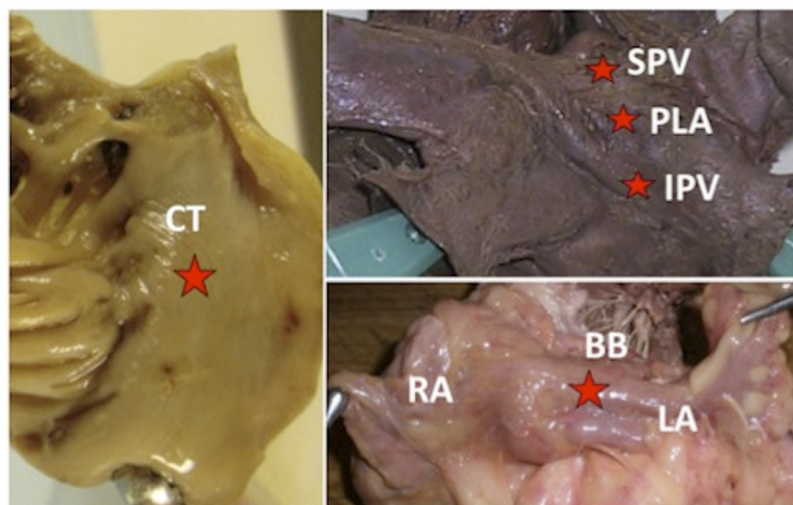


Figure 1. Tissue Sampling Locations

Locations of tissue sample collection (red stars) from crista terminalis at lateral right atrium (CT), Bachmann's bundle (BB), and posterior left atrial wall (PLA) at 3 locations between superior pulmonary veins (SPV), inferior pulmonary veins (IPV), and in the center of PLA. LA = left atrium; RA = right atrium.

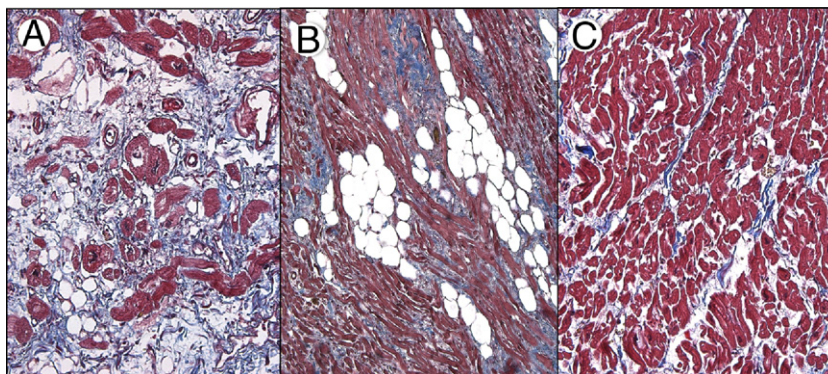


Figure 2 Fibrosis Extent in Atrial Walls

Light microscopy of crista terminalis specimens in patients with (A) permanent atrial fibrillation (AF), (B) paroxysmal atrial fibrillation, and (C) without history of AF. (A) Fibrosis extent 51%, fat 15%, capillary density 2%, mean cardiomyocyte diameter 12 μm . (B) Fibrosis extent 14%, fat 24%, capillary density 0.4%, mean cardiomyocyte diameter 11 μm . (C) Fibrosis extent 5%, fat 1%, capillary density 1%, mean cardiomyocyte diameter 15 μm . (Masson's trichrome stain; original magnification $\times 200$.)

Myocardial infiltration of inflammatory cells was examined histologically using polyclonal rabbit anti-human antibodies to CD3 pan-T cellular antigen (Dako Denmark A/S, Glostrup, Denmark) and monoclonal mouse anti-human antibodies to CD45 leukocyte common antigen clone 2B11 + PD7/26 (Dako Denmark A/S). Immunostaining was carried out in 3 samples taken at CT, BB, and PLA. Inflammatory cell count was performed separately for CD3-positive and CD45-positive cells at $\times 200$ magnification and calculated as a mean cell count per 1 mm^2 at 10 fields of view at each location.

Statistical analyses. Data are expressed as the mean \pm SD unless stated otherwise. All statistical analyses were performed using IBM SPSS Statistics, version 19.0.0 (SPSS, Chicago, Illinois). An independent-samples (Kruskal-Wallis) test was used for comparing continuous variables among the 3 patient groups. The Mann-Whitney test was used for 2-group comparisons. No correction for multiple testing was applied. Spearman's correlation coefficient was calculated for analyzing correlation between quantified histological variables and clinical characteristics. All tests were 2 sided, and $p < 0.05$ was considered statistically significant.

Results

Patient characteristics. Clinical characteristics of patients are presented in Table 1. Median age was 62 years (range 39 to 86 years). The majority of patients died of cardiovascular causes such as acute myocardial infarction ($n = 20$), stroke ($n = 1$), and pulmonary embolism ($n = 7$). Two patients died of pneumonia.

Patients without a history of AF were older than patients with any history of AF (68 ± 10 years vs. 62 ± 13 years) and had an over-representation of men (8 of 10 vs. 9 of 20); however, these differences did not reach statistical significance. There was no difference in regard to the presence of ischemic heart disease, hypertension, stroke, chronic ob-

structive pulmonary disease, diabetes mellitus, or aortic or mitral valve pathology between patients with AF history and without AF history. Comorbidity status expressed in CHA₂DS₂-VASc score was 3.8 ± 1.8 in AF patients versus 4.3 ± 1.9 in patients without AF ($p = 0.05$). CHADS₂ score was 2.6 ± 1.3 versus 2.8 ± 1.2 in AF and non-AF patients, respectively ($p = 0.7$).

Table 1 Patient Characteristics

Characteristics	AF History		
	No AF (n = 10)	Paroxysmal AF (n = 10)	Permanent AF (n = 10)
Age, yrs	68 \pm 10	62 \pm 10	61 \pm 15
Male	8	4	5
BMI, kg/m ²	26.4 \pm 7.1	28.7 \pm 6.8	23.4 \pm 6.8
Left atrial diameter, mm	40 \pm 5	48 \pm 8	56 \pm 13*
LVEF, %	39 \pm 15	40 \pm 19	41 \pm 7
QRS \geq 120 ms	3	5	1
Comorbidities			
Ischemic heart disease	10	7	9
CHF	10	9	10
Hypertension	8	9	8
Diabetes mellitus	3	1	4
Stroke	3	2	3
COPD	4	2	0
CHA ₂ DS ₂ -VASc score	4.3 \pm 1.9	3.3 \pm 1.5	4.3 \pm 1.9
CHADS ₂ score	2.8 \pm 1.2	2.4 \pm 1.2	2.9 \pm 1.4
Pharmacological therapy			
ACEI or ARB	10	8	10
Statins	6	3	3
Amiodarone	0	3	3
Beta-blockers	8	9	10
Digoxin	1	0	6†

Values are mean \pm SD or n. * $p = 0.026$ in comparison with no atrial fibrillation (AF) group. † $p = 0.004$ in comparison with no AF and paroxysmal AF groups.

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin-2 receptor blocker; BMI = body mass index; COPD = chronic obstructive pulmonary disease; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction.

There was no difference between the groups in regard to pharmacological treatment with angiotensin-converting enzyme inhibitors, angiotensin-2 receptor blockers, beta-blockers, statins, or diuretics. Digoxin was commonly used in PerMAF patients but not in the NoAF or PxAF groups (Table 1).

Echocardiographic data were available for 21 patients (16 AF and 5 NoAF patients) and concerned examinations performed during these patients' last hospital admission. The LA diameter measured in parasternal view was significantly larger in patients with AF history (52 ± 11 mm vs. 40 ± 5 mm, $p = 0.039$) while there was no difference in left ventricular ejection fraction ($39 \pm 15\%$ vs. $40 \pm 14\%$, $p = 0.802$).

Medical record information on AF history duration (median 24 months, range 0.5 to 192 months) was available for 18 patients. The remaining 2 patients presented with PxAF with unknown time of symptom onset.

Site-dependent histological abnormalities. The extent of fibrosis, fatty infiltration, capillary density, and cardiomyocyte size did not differ among the 5 sampling locations in the atria, neither in total material nor in subgroup analysis (data not shown).

Possible correlation between fibrosis and any other histological variable was assessed at all 5 locations. The extent of fibrosis demonstrated weak positive correlation with the mean cardiomyocyte diameter at BB ($r = 0.589$, $p = 0.001$), IPV ($r = 0.459$, $p = 0.011$), and PLA ($r = 0.453$, $p = 0.012$) locations. We observed no significant correlation between the extent of fibrosis and capillary density at any location. In PLA, the extent of fibrosis showed significant positive correlation with the extent of fatty infiltration ($r = 0.439$, $p = 0.015$), but at all other locations, no such association was observed (data not shown).

The extent of fibrosis positively correlated with CD3-positive and CD45-positive leukocyte count at BB ($r = 0.75$, $p < 0.001$ for CD3; and $r = 0.67$, $p < 0.001$ for CD45) and PLA ($r = 0.76$, $p < 0.001$ for CD3; and $r = 0.67$, $p < 0.001$ for CD45), but not at CT.

Atrial fibrillation history. Patients with any history of AF had a 3- to 5-fold greater extent of fibrosis and fatty tissue compared to patients without AF history, regardless of tissue sampling location (Table 2). Similarly, a strong association between inflammatory cell count and AF history was observed at all tissue sampling locations where inflammatory response was assessed (Table 3). However, neither capillary density nor mean cardiomyocyte diameter showed any notable difference among the groups in regard to the AF history.

A 3-group comparison revealed generally greater extent of fibrosis in PerMAF patients compared with PxAF patients, and passed the significance threshold for BB and SPV locations (Fig. 3). This phenomenon, however, was not observed when the extent of fatty tissue was compared.

The extent of fibrosis alone showed borderline significance for correlation with AF history duration at IPV ($r =$

Table 2 Fibrosis and Fatty Tissues in Relation to Age and AF History

	Age		AF History	
	<60 Yrs (n = 11)	≥60 Yrs (n = 19)	No AF (n = 10)	AF (n = 20)
Fibrosis, %				
Crista terminalis	23 ± 19	20 ± 14	8 ± 5	28 ± 15†
Bachmann's bundle	21 ± 16	18 ± 14	8 ± 4	25 ± 14†
Inferior PV	28 ± 23	13 ± 10	5 ± 3	26 ± 18†
Posterior LA	26 ± 17	14 ± 11*	6 ± 3	25 ± 13†
Superior PV	23 ± 16	14 ± 10	7 ± 5	22 ± 13†
Fatty tissue, %				
Crista terminalis	8 ± 7	9 ± 10	3 ± 5	12 ± 10‡
Bachmann's bundle	13 ± 13	7 ± 4	4 ± 4	13 ± 10‡
Inferior PV	10 ± 11	7 ± 7	4 ± 4	8 ± 5*
Posterior LA	7 ± 5	6 ± 5	3 ± 3	8 ± 5‡
Superior PV	9 ± 11	9 ± 9	4 ± 3	12 ± 10‡
Fibrosis and fatty tissue, %				
Crista terminalis	32 ± 22	29 ± 18	12 ± 7	40 ± 17†
Bachmann's bundle	34 ± 19	26 ± 17	11 ± 5	38 ± 16†
Inferior PV	37 ± 26	20 ± 15	9 ± 5	35 ± 20†
Posterior LA	33 ± 20	20 ± 13	8 ± 4	33 ± 15†
Superior PV	32 ± 18	23 ± 15	11 ± 4	34 ± 15†
Capillary density, %				
Crista terminalis	1.6 ± 1.0	1.5 ± 0.7	1.2 ± 0.7	1.4 ± 0.8
Bachmann's bundle	2.5 ± 1.4	1.8 ± 1.9	2.4 ± 2.5	1.9 ± 1.3
Inferior PV	2.9 ± 1.2	1.8 ± 2.5	2.9 ± 3.3	1.8 ± 1.4
Posterior LA	1.8 ± 0.8	1.6 ± 1.2	2.0 ± 1.0	1.6 ± 1.0
Superior PV	1.6 ± 0.9	2.2 ± 2.4	1.0 ± 0.3	2.0 ± 2.4
Cardiomyocyte diameter, μm				
Crista terminalis	20 ± 4	18 ± 4	18 ± 4	19 ± 4
Bachmann's bundle	20 ± 8	20 ± 4	18 ± 4	21 ± 6
Inferior PV	20 ± 5	19 ± 3	17 ± 3	20 ± 4
Posterior LA	19 ± 5	20 ± 4	18 ± 3	20 ± 5
Superior PV	21 ± 4	19 ± 4	19 ± 3	20 ± 4

Values are mean ± SD. * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.
AF = atrial fibrillation; LA = left atrium; PV = pulmonary vein.

0.466 , $p = 0.05$) (Fig. 4), but not at any other location. The correlation of combined count for fibrosis and fatty infiltration and AF history duration at IPV was stronger than for fibrosis alone ($r = 0.675$, $p = 0.002$) (Fig. 4). The AF history duration also showed positive correlation with cardiomyocyte diameter that reached significance at BB location ($r = 0.542$, $p = 0.02$).

Left atrial diameter. Left atrial diameter strongly correlated with the extent of fibrosis at all LA locations, IPV ($r = 0.548$, $p = 0.01$), PLA ($r = 0.574$, $p = 0.006$), and SPV ($r = 0.687$, $p = 0.001$), whereas fatty infiltration showed positive correlation with LA diameter at BB ($r = 0.490$, $p = 0.024$), CT ($r = 0.496$, $p = 0.026$), and PLA ($r = 0.502$, $p = 0.02$). Combined fibrosis and fatty tissue count correlated with LA diameter at all 5 locations: CT ($r = 0.504$, $r = 0.020$), BB ($r = 0.610$, $p = 0.003$), IPV ($r = 0.618$, $p = 0.003$), PLA ($r = 0.634$, $p = 0.002$), and SPV ($r = 0.598$, $p = 0.004$). Neither capillary density nor mean cardiomyocyte diameter was associated with LA diameter.

Table 3 Inflammatory Cell Count in Major Atrial Pathways and Posterior Left Atrial Wall in Patients With and Without AF History

	No AF (n = 10)	Paroxysmal AF (n = 10)	Permanent AF (n = 10)	Total AF (n = 20)
CD45+ cell count				
Crista terminalis	10.6 ± 4.4	23.5 ± 6.9*	25.7 ± 7.3‡	24.6 ± 7.0‡
Bachmann's bundle	9.3 ± 4.5	20.7 ± 4.1†	28.8 ± 11.4‡	24.8 ± 9.3‡
Posterior left atrial wall	9.8 ± 4.5	19.9 ± 3.8*	25.6 ± 6.9‡	22.7 ± 6.2‡
CD3+ cell count				
Crista terminalis	7.6 ± 4.4	19.2 ± 7.8*	24.4 ± 7.5‡	21.8 ± 7.9‡
Bachmann's bundle	5.2 ± 3.2	16.6 ± 6.2†	27.3 ± 11.9‡	21.9 ± 10.7‡
Posterior left atrial wall	4.2 ± 2.0	14.6 ± 5.4†	25.1 ± 8.6‡	19.9 ± 8.8‡

Values are mean ± SD. *p < 0.05; †p < 0.01; ‡p < 0.001 in comparison with no atrial fibrillation (AF) group.

The CD3-positive cell count demonstrated positive correlation with LA diameter at all tissue sampling locations: $r = 0.45, 0.50,$ and 0.47 for CT, BB, and PLA, respectively (all $p < 0.05$). The CD45-positive cell count correlated with LA diameter at CT and BB ($r = 0.46$ and 0.47 , respectively, $p < 0.05$ for both), but not at PLA.

Age and sex. No sex-related associations were observed for any histology parameter. No increase in atrial fibrosis associated with higher age was observed either in total material or in patient subgroups with or without AF history (Fig. 3). None of the histology parameters, including fibrosis, showed any significant correlation with age in either subgroups or total material (data not shown) using Spearman's correlation.

Discussion

Our study, carried out in a systematic fashion, provides histological evidence of a robust association between extent of structural changes in left atrial walls and major atrial conduction pathways with AF history and clinical type. Patients with permanent AF consistently had more extensive fibrofatty replacement of atrial myocardium than patients with paroxysmal AF and otherwise similar clinical profile. On the contrary, despite the wide age range and the presence of comorbidities likely to contribute to fibrosis development in all 3 groups, we were not able to detect any correlation between patient age and increase in the extent of fibrosis or fatty changes in atrial tissue, in either total material or subgroup analyses.

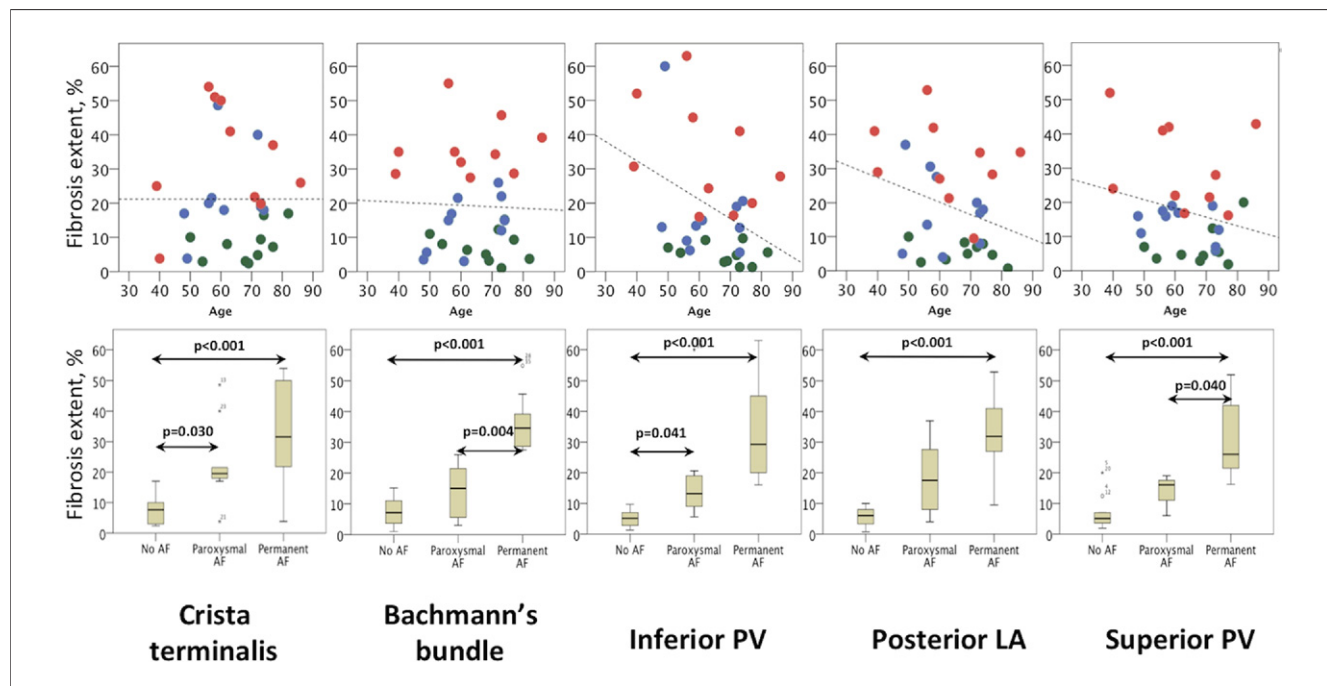


Figure 3 Fibrosis Extent, Age, and Clinical Type of AF

Association between fibrosis extent, age (upper panels) and atrial fibrillation (AF) type (lower panels) at all tissue sampling locations. Red circles = permanent AF; blue circles = paroxysmal AF; green circles = no AF. The extent of fibrosis is significantly associated with clinical type of AF but not associated with age. Whisker lengths in the box plots correspond to ranges in the data series. LA = left atrium; PV = pulmonary vein.

Previous studies. Human data on association between the extent of structural atrial changes and the presence of lone AF are scarce. In 1 study (9), the presence of patchy fibrosis along with widespread inflammatory changes was observed in atrial septal biopsies taken from patients with lone AF but not in patients without AF history. However, it was unclear whether AF in patients with cardiovascular disease would demonstrate a similarly strong association between fibrosis and arrhythmia or whether structural abnormalities confined to the atrial septum were also present in other parts of atria. Our study of nonvalvular AF in patients with comorbidities commonly associated with development of AF, namely, mainly ischemic heart disease and hypertension, is relevant to the vast majority of AF patients seen in clinical practice.

Absolute values of fibrosis extent observed in the NoAF group are in the same range as those reported in right atrial appendage biopsies collected from patients without AF history during open-heart surgery using similar fibrosis quantification techniques (10,11,13). Swartz et al. (11) examined tissue samples from right and left atrial appendages collected from 44 patients without prior AF history during open-heart surgery and reported $10.8 \pm 11\%$ fibrosis extent in patients who had post-operative AF compared with $3.8 \pm 3.5\%$ in patients remaining in normal sinus rhythm. The authors also reported more fibrosis in left atrium compared with right atrium, but that finding is not supported by our study. In a study by Goette et al. (10), fibrosis extent in right atrial appendage was significantly associated with both age and AF incidence in the post-operative period, thus supporting the causative role of age-related fibrosis in post-operative AF. The discrepancy can be explained by significant differences between post-operative AF in the study by Swartz et al. (11), whereas AF was remote from open-heart surgery in our cases.

Our finding of a strong association between fibrosis extent and inflammatory cell count in patients with AF history is in agreement with earlier reports (14,15), and further supports the important role inflammation plays in creation of an AF substrate. We demonstrated the presence of inflammatory infiltrate in major atrial pathways (CT and BB) and in posterior LA wall in patients with AF, and documented its association with LA dilation.

Fibrosis in AF: cause or consequence? Recent developments in imaging and the introduction of delayed-enhancement technology provided indirect evidence of fibrosis in the left atrium associated with the presence of AF regardless of comorbidities (7). In another study, delayed-enhancement magnetic resonance imaging revealed that patients with persistent AF compared with paroxysmal AF showed more fibrosis in the left atrium (16). Our study is in agreement with these observations and provides direct histological evidence of the pathophysiological association between extent of fibrofatty atrial abnormalities and AF presence and persistency.

Our study results, however, do not answer the question of whether the fibrofatty replacement of atrial myocardium associated with AF presence is the cause or the consequence of the arrhythmia. The observed structural alterations in the patients in our study could have developed as a result of more or less long-standing AF, and, if so, the changes we observed truly represent structural atrial remodeling. However, fibrosis may have occurred secondary to an unknown factor such as an inflammatory process in the atrial myocardium or an inherited predisposition to fibrosis development. Numerous observations of increased inflammatory state markers in patients with AF (17) and our findings of the close link between fibrosis extent and lymphomononuclear infiltration support this hypothesis. Several genetic loci associated with AF have been reported recently in large-

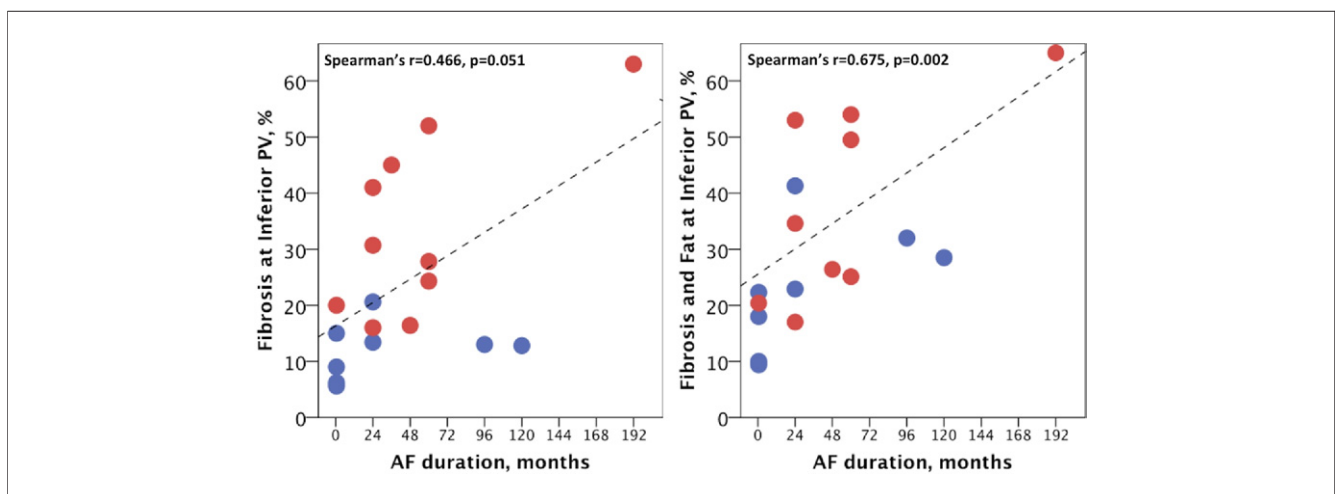


Figure 4 Association Between Fibrosis, Fat, and Duration of AF History

Extent of fibrosis (left) and combined fibrosis and fat (right) in the vicinity of inferior pulmonary veins (PV) demonstrate positive correlation with atrial fibrillation (AF) history duration. Blue circles = paroxysmal AF; red circles = permanent AF.

scale epidemiological studies; however, their relation to the development of fibrosis is not yet known (18–20).

Experimental data comparing AF promoted by rapid atrial pacing and the development of congestive heart failure in dogs suggest that atrial pacing-induced AF in the absence of congestive heart failure per se does not promote development of fibrosis (1). Our findings in humans contradict these experimental data. First, we were not able to detect any link between the extent of fibrosis and left ventricular ejection fraction, whereas the significant association between LA size and structural abnormalities mostly confined to LA was rather an expected finding. Second, atrial samples taken from age-matched arrhythmia-free patients contained negligibly low amounts of fibrofatty tissue despite similar clinical profile and the presence of congestive heart failure, ischemic heart disease, or hypertension in the majority of subjects in all 3 groups. It cannot be completely ruled out that patients without AF history in our study had less severe clinical manifestations of accompanying conditions, leading to less advanced atrial structural changes that could not be reliably evaluated in a retrospective journal-based analysis. Nevertheless, patients with permanent AF in our study had significantly more extensive structural atrial abnormalities than did patients with paroxysmal AF associated with AF history duration.

Structural abnormalities of major conduction pathways. We have demonstrated not only the presence of fibrofatty replacement of atrial myocardium in the LA recently linked to the effect of catheter ablation (7), but we also analyzed the extent of structural abnormalities in major atrial conduction pathways, namely, BB and CT. Deteriorated atrial conduction in patients with paroxysmal AF has been well documented in electrocardiographic (21,22) and invasive electrophysiological studies (23,24). In a recent study of patients enrolled in the MADIT-II (Multicenter Automatic Defibrillator Implantation Trial II), we have shown that abnormal atrial conduction bears an independent predictive value for developing new-onset AF in patients with ischemic heart failure, thus supporting the primary role of conduction defects in developing AF in this patient population (25). Although the possibility of strategically located structural atrial abnormalities affecting the BB and terminal crest was reported in an earlier study by Becker et al. (26) on both valvular and nonvalvular AF, our study provides a morphometric analysis of these changes and suggests that the changes are likely to represent a manifestation of a generalized pathological process affecting the LA and right atrium equally.

Lack of age-related increase in extent of structural changes. Although an association between age and atrial fibrosis has become conventional wisdom, and “age-related fibrosis” is listed as 1 of the etiological factors underlying AF development in management guidelines (4), analyses of atrial wall histology, with few exceptions (8,9), were mostly performed on patients undergoing open-heart surgery without prior history of AF (10,11,13). Prevalence of AF has

been clearly shown to increase with age (27,28); however, whether the increase was due to extensive atrial fibrosis has not been convincingly demonstrated. In a recent report, right atrial appendage biopsies taken during open-heart surgery in patients without AF history revealed positive correlation between fibrosis extent and age (13). Our study found no such correlation in a similar age span (although smaller) population, suggesting that age-related increase in fibrosis extent does not reach the magnitude of changes observed in AF. We propose that age contribution to fibrosis development is limited and unlikely to be the sole explanation of the advanced fibrosis observed in AF patients.

Study limitations. We had to rely on medical record data without the possibility of additional investigations to resolve uncertainties or verify AF diagnosis. For the same reason, echocardiographic variables are limited to left ventricular ejection fraction and LA diameter as other measurements relevant in the context of atrial remodeling, including left ventricular diastolic function assessment, were not consistently reported for all subjects. Finally, the small number of cases might lead to possible underestimation of age effect on structural changes of atrial walls, but we believe our detailed histological analysis could mitigate that.

Conclusions

In post-mortem atrial tissues from patients who died of cardiovascular causes, the extent of fibrosis was not associated with age, but was significantly correlated with AF presence, severity, and duration. Chronic inflammation in atrial myocardium is likely to play an important role in this process. Our findings suggest that age-related changes per se are unlikely to be the sole cause of advanced fibrosis underlying AF.

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- Key Words:** atrial fibrillation ■ fibrosis ■ inflammation ■ structural remodeling.