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# Structural abnormalities in atrial walls are associated with presence and persistency of atrial fibrillation but not with age

*Atrial fibrosis in atrial fibrillation*

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## **Abstract**

**Objective:** To assess 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 (age  $64 \pm 12$  y) were collected in three equal age-matched groups as patients without AF history, with paroxysmal or permanent AF. Tissue samples were obtained at the level of superior pulmonary veins, inferior pulmonary veins (IPV), center of posterior left atrial wall, terminal crest and Bachmann's bundle. Histological 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 two- 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 those with paroxysmal AF.

**Conclusion:** In *post-mortem* material, structural changes in the atria were not associated with age but 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.

**Key words:** atrial fibrillation, fibrosis, inflammation, structural remodeling

## List of abbreviations

ACE	angiotensin-converting enzyme
AF	atrial fibrillation
BB	Bachmann's bundle
CT	crista terminalis
DE-MRI	delayed enhancement magnetic resonance imaging
IPV	inferior pulmonary veins
LA	left atrium
PLA	posterior left atrial wall
SPV	superior pulmonary veins

## Introduction

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 development of methodology aimed at detecting the presence of atrial fibrosis with delayed enhancement by using extracellular gadolinium-based contrast agents (DE-MRI)(5). Using this technique, the presence and extent of atrial fibrosis showed an association with AF persistency(6) 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 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 non-valvular AF is lacking.

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

## **Material**

### ***Study design***

Following 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. In order 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 three 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 one year prior to death (PermAF group).

### ***Tissue sample collection and handling***

Transmural atrial tissue samples of at least 20 x 3 mm were collected from five locations that included major atrial conduction pathways and posterior LA region in the vicinity of pulmonary vein ostia (Figure 1). The sites were: crista terminalis at right atrium lateral wall (CT); Bachmann's bundle from the superior portion of the interatrial groove between the atria (BB); posterior LA wall at superior pulmonary veins (SPV) level; centrally between pulmonary vein ostia (PLA); 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 (Figure 2).

Specimens were examined with computer-assisted morphometric analysis using the Leica LAS Image Analysis System (LeicaQWin Plus v3, Leica Microsystems Imaging Solutions Ltd, Cambridge, UK). The percentage of fatty infiltration, interstitial fibrosis, capillary density and mean cardiomyocyte diameter from within each sample were assessed at 200x magnification and calculated at ten 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 ten measurements for each parameter per location was used for further analysis.

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 leucocyte common antigen clone 2B11 + PD7/26 (Dako Denmark A/S, Glostrup, Denmark). Immunostaining was carried out in three samples taken at CT, BB and PLA. Inflammatory cell count was performed separately for CD3- and CD45-positive cells at 200x magnification and calculated as a mean cell count per 1 mm<sup>2</sup> at ten fields of view at each location.

### ***Statistical analyses***

Data are expressed as the mean ± standard deviation unless stated otherwise. All statistical analyses were performed using IBM SPSS Statistics 19, version 19.0.0. An independent samples (Kruskal-Wallis) test was used for comparing continuous variables between the three patient groups. The Mann-Whitney test was used for two-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 two-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 39-86 years). Majority of patients died from cardiovascular causes such as acute myocardial infarction (n=20), stroke (n=1) and pulmonary embolism (n=7). Two patients died from pneumonia.

Patients without history of AF were older than patients with any history of AF ( $68\pm 10$  vs.  $62\pm 13$  years) and had overrepresentation of men (8/10 vs. 9/20); however, these differences did not reach statistical significance. There was no difference in regard to the presence of ischemic heart disease, hypertension, stroke, chronic obstructive pulmonary disease, diabetes mellitus or aortic or mitral valve pathology between patients with and without AF history. Comorbidity status expressed in CHA<sub>2</sub>DS<sub>2</sub>-VASc score was  $3.8\pm 1.8$  in AF patients vs  $4.3\pm 1.9$  in patients without AF ( $p=0.5$ ). CHADS<sub>2</sub> - score was  $2.6\pm 1.3$  vs  $2.8\pm 1.2$  in AF and NoAF patients respectively ( $p=0.7$ ).

There was no difference between the groups in regard to pharmacological treatment with ACE-inhibitors, angiotensin-2 receptor blockers, beta-blockers, statins or diuretics. Digoxin was commonly used in PermAF patients but not in 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. LA diameter measured in parasternal view was significantly larger in patients with AF history ( $52\pm 11$  vs  $40\pm 5$  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 was available for 18 patients (median 24 months, range 0.5-192 months). The remaining two 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 five 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 five 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- and CD45-positive leucocyte 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 three- to five- 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 three-group comparison revealed generally greater extent of fibrosis in patients with PermAF compared with PxAF patients that passed the significance threshold for BB and SPV locations (Figure 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 ( $r=0.466$ ,  $p=0.05$ ), 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 ( $r=0.675$ ,  $p=0.002$ ). 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***

LA diameter strongly correlated with the extent of fibrosis at all LA locations (IPV:  $r=0.548$ ,  $p=0.01$ ; PLA:  $r=0.574$ ,  $p=0.006$ ; SPV:  $r=0.687$ ,  $p=0.001$ ) while 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 five locations (CT:  $r=0.504$ ,  $p=0.020$ ; BB:  $r=0.610$ ,  $p=0.003$ ; IPV:  $r=0.618$ ,  $p=0.003$ ; PLA:  $r=0.634$ ,  $p=0.002$ ; SPV:  $r=0.598$ ,  $p=0.004$ ). Neither capillary density nor mean cardiomyocyte diameter were associated with LA diameter.

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  $p<0.05$ ). 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 gender***

No gender-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 (Figure 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 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 fibro-fatty 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 three 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.

### ***Previous studies***

Human data on association between the extent of structural atrial changes and the presence of lone AF are scarce. In one 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 those 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 non-valvular AF in patients with comorbidities commonly associated with development of AF, i.e. 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 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 technique(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 developed postoperative AF compared with  $3.8\% \pm 3.5\%$  in those remaining in normal sinus rhythm. The authors also reported more fibrosis in left atrium compared with right atrium, but the 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 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 postoperative AF in the Swartz et al.(11) study, 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 dilatation.

### ***Fibrosis in atrial fibrillation: 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, DE-MRI 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 pathophysiological association between extent of fibro-fatty atrial abnormalities and AF presence and persistency.

Our study results, however, do not answer the question of whether the fibro-fatty replacement of atrial myocardium associated with AF presence is the cause or the consequence of the arrhythmia. The patients in our study could have developed the observed structural alterations 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-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 atrial fibrillation 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, while 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 fibro-fatty tissue despite similar clinical profile and the presence of congestive heart failure, ischemic heart disease or hypertension in the majority of subjects in all three 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 patients with paroxysmal AF associated with AF history duration.

#### ***Structural abnormalities of major conduction pathways***

We have demonstrated not only the presence of fibro-fatty replacement of atrial myocardium in the left atrium recently linked to the effect of catheter ablation(7) but we also analyzed the extent of structural abnormalities in major atrial conduction pathways namely Bachmann's bundle and crista terminalis. 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 on patients enrolled in the MADIT-II trial, 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 Bachmann's bundle and terminal crest was reported in an earlier study by Becker et al. on both valvular and non-valvular AF(26), 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 left and right atria equally.

#### ***Lack of age-related increase in extent of structural changes***

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

### **Limitations**

We had to rely on medical record data without possibility of additional investigations in order 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 in 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.

### **Conclusion**

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 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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## References

1. Li D, Fareh S, Leung TK, Nattel S. Promotion of atrial fibrillation by heart failure in dogs: atrial remodeling of a different sort. *Circulation* 1999;100:87-95.
2. Morillo CA, Klein GJ, Jones DL, Guiraudon CM. Chronic rapid atrial pacing. Structural, functional, and electrophysiological characteristics of a new model of sustained atrial fibrillation. *Circulation* 1995;91:1588-95.
3. Anyukhovskiy EP, Sosunov EA, Plotnikov A, et al. Cellular electrophysiologic properties of old canine atria provide a substrate for arrhythmogenesis. *Cardiovasc Res* 2002;54:462-9.
4. Camm AJ, Kirchhof P, Lip GY, et al. Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology. *Eur Heart J* 2010;31:2369-429.
5. McGann CJ, Kholmovski EG, Oakes RS, et al. New magnetic resonance imaging-based method for defining the extent of left atrial wall injury after the ablation of atrial fibrillation. *J Am Coll Cardiol* 2008;52:1263-71.
6. Oakes RS, Badger TJ, Kholmovski EG, et al. Detection and quantification of left atrial structural remodeling with delayed-enhancement magnetic resonance imaging in patients with atrial fibrillation. *Circulation* 2009;119:1758-67.
7. Mahnkopf C, Badger TJ, Burgon NS, et al. Evaluation of the left atrial substrate in patients with lone atrial fibrillation using delayed-enhanced MRI: implications for disease progression and response to catheter ablation. *Heart Rhythm* 2010;7:1475-81.
8. Corradi D, Callegari S, Benussi S, et al. Myocyte changes and their left atrial distribution in patients with chronic atrial fibrillation related to mitral valve disease. *Hum Pathol* 2005;36:1080-9.
9. Frustaci A, Chimenti C, Bellocci F, Morgante E, Russo MA, Maseri A. Histological substrate of atrial biopsies in patients with lone atrial fibrillation. *Circulation* 1997;96:1180-4.



10. Goette A, Juenemann G, Peters B, et al. Determinants and consequences of atrial fibrosis in patients undergoing open heart surgery. *Cardiovasc Res* 2002;54:390-6.
11. Swartz MF, Fink GW, Lutz CJ, et al. Left versus right atrial difference in dominant frequency, K(+) channel transcripts, and fibrosis in patients developing atrial fibrillation after cardiac surgery. *Heart Rhythm* 2009;6:1415-22.
12. Mariscalco G, Engstrom KG, Ferrarese S, et al. Relationship between atrial histopathology and atrial fibrillation after coronary bypass surgery. *J Thorac Cardiovasc Surg* 2006;131:1364-72.
13. Gramley F, Lorenzen J, Knackstedt C, et al. Age-related atrial fibrosis. *Age* 2009;31:27-38.
14. Chen MC, Chang JP, Liu WH, et al. Increased inflammatory cell infiltration in the atrial myocardium of patients with atrial fibrillation. *Am J Cardiol* 2008;102:861-5.
15. Yamashita T, Sekiguchi A, Iwasaki YK, et al. Recruitment of immune cells across atrial endocardium in human atrial fibrillation. *Circ J* 2010;74:262-70.
16. Kuppahally SS, Akoum N, Burgon NS, et al. Left atrial strain and strain rate in patients with paroxysmal and persistent atrial fibrillation: relationship to left atrial structural remodeling detected by delayed-enhancement MRI. *Circ Cardiovasc Imaging* 2010;3:231-9.
17. Chung MK, Martin DO, Sprecher D, et al. C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanisms and persistence of atrial fibrillation. *Circulation* 2001;104:2886-91.
18. Gudbjartsson DF, Arnar DO, Helgadottir A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature* 2007;448:353-7.
19. Benjamin EJ, Rice KM, Arking DE, et al. Variants in ZFX3 are associated with atrial fibrillation in individuals of European ancestry. *Nat Genet* 2009;41:879-81.
20. Sinner MF, Pfeufer A, Akyol M, et al. The non-synonymous coding IKr-channel variant KCNH2-K897T is associated with atrial fibrillation: results from a systematic candidate gene-based analysis of KCNH2 (HERG). *Eur Heart J* 2008;29:907-14.

21. Bayes de Luna A, Cladellas M, Oter R, et al. Interatrial conduction block and retrograde activation of the left atrium and paroxysmal supraventricular tachyarrhythmia. *Eur Heart J* 1988;9:1112-8.
22. Platonov PG, Carlson J, Ingemansson MP, et al. Detection of inter-atrial conduction defects with unfiltered signal-averaged P-wave ECG in patients with lone atrial fibrillation. *Europace* 2000;2:32-41.
23. Platonov PG, Yuan S, Hertervig E, et al. Further evidence of localized posterior interatrial conduction delay in lone paroxysmal atrial fibrillation. *Europace* 2001;3:100-7.
24. Cosio FG, Palacios J, Vidal JM, Cocina EG, Gomez-Sanchez MA, Tamargo L. Electrophysiologic studies in atrial fibrillation. Slow conduction of premature impulses: a possible manifestation of the background for reentry. *Am J Cardiol* 1983;51:122-130.
25. Holmqvist F, Platonov PG, Carlson J, Zareba W, Moss AJ. Altered interatrial conduction detected in MADIT II patients bound to develop atrial fibrillation. *Ann Noninvasive Electrocardiol* 2009;14:268-75.
26. Becker AE. How structurally normal are human atria in patients with atrial fibrillation? *Heart Rhythm* 2004;1:627-31.
27. Kannel WB, Abbott RD, Savage DD, McNamara PM. Epidemiologic features of chronic atrial fibrillation: the Framingham study. *N Engl J Med* 1982;306:1018-22.
28. Smith JG, Platonov PG, Hedblad B, Engstrom G, Melander O. Atrial fibrillation in the Malmo Diet and Cancer study: a study of occurrence, risk factors and diagnostic validity. *Eur J Epidemiol*;25:95-102.

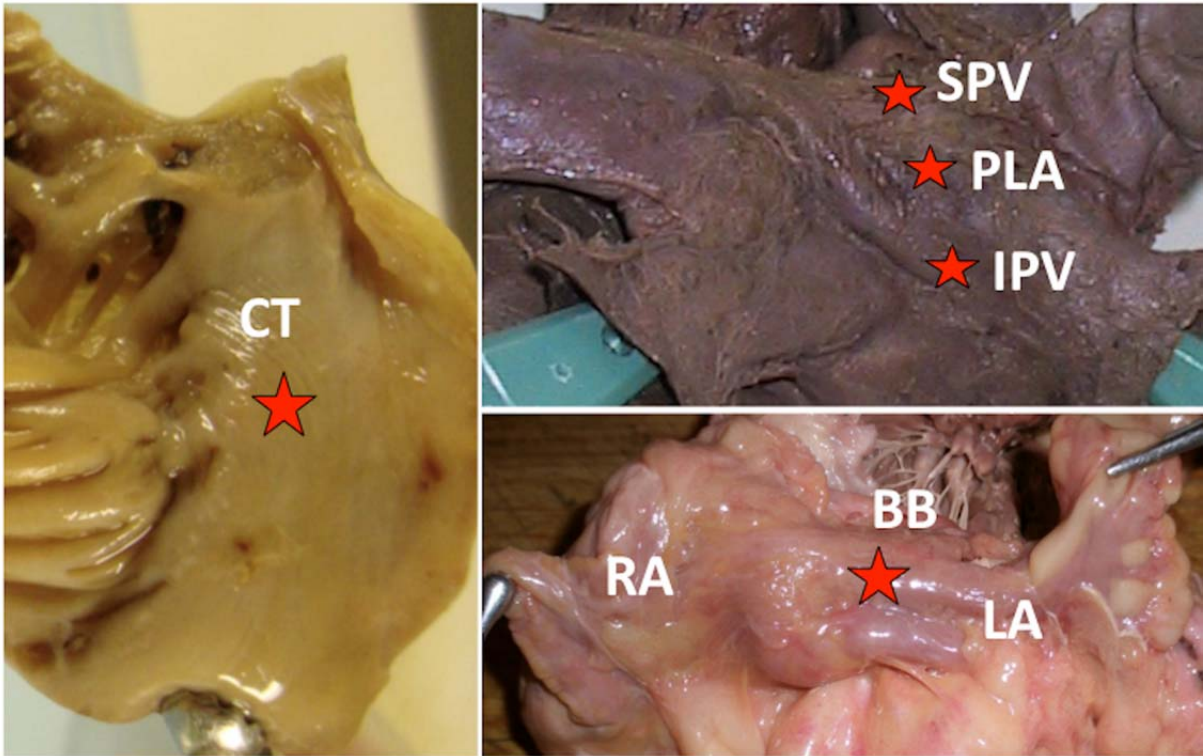


Figure 1: Tissue sampling locations

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

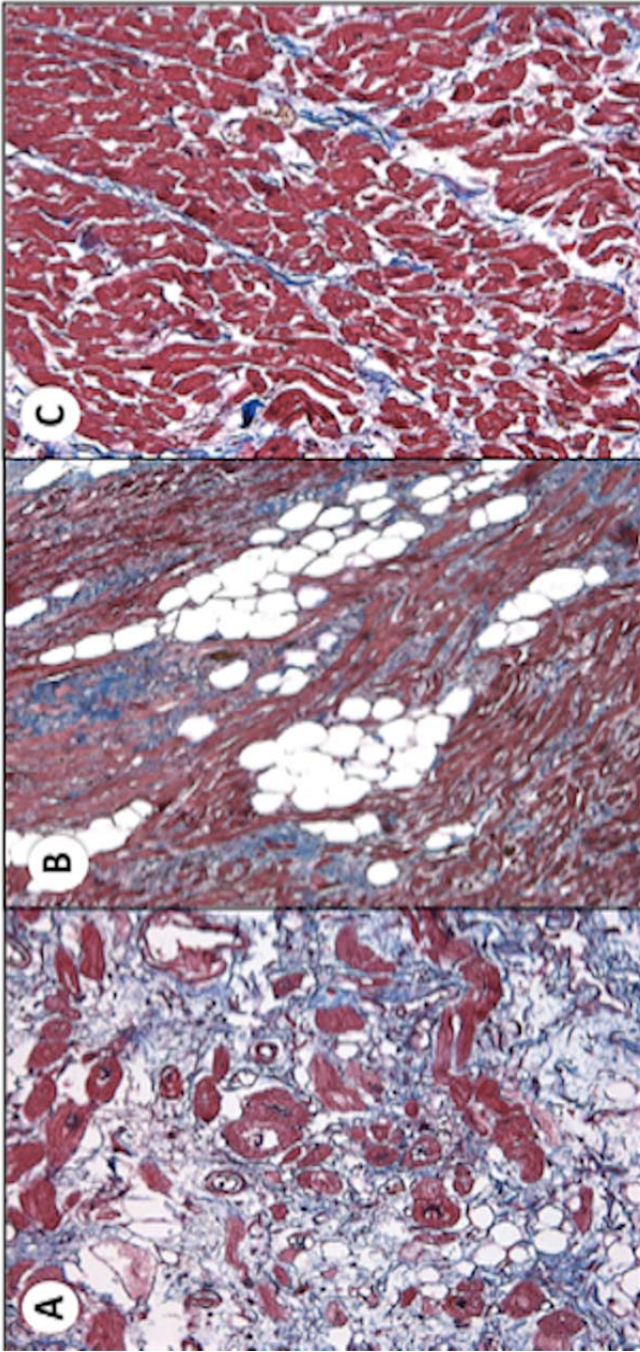


Figure 2: Fibrosis extent in atrial walls

Light microscopy of crista terminalis specimens in patients with permanent atrial fibrillation (A), paroxysmal atrial fibrillation (B) and without history of AF (C). A: Fibrosis extent 51%, fat 15%, capillary density 2%, mean cardiomyocyte diameter 12 $\mu$ m; B: Fibrosis extent 14%, fat 24%, capillary density 0,4%, mean cardiomyocyte diameter 11 $\mu$ m; C: Fibrosis extent 5%, fat 1%,capillary density 1%, mean cardiomyocyte diameter 15  $\mu$ m. Masson's trichrome stain, x200 magnification

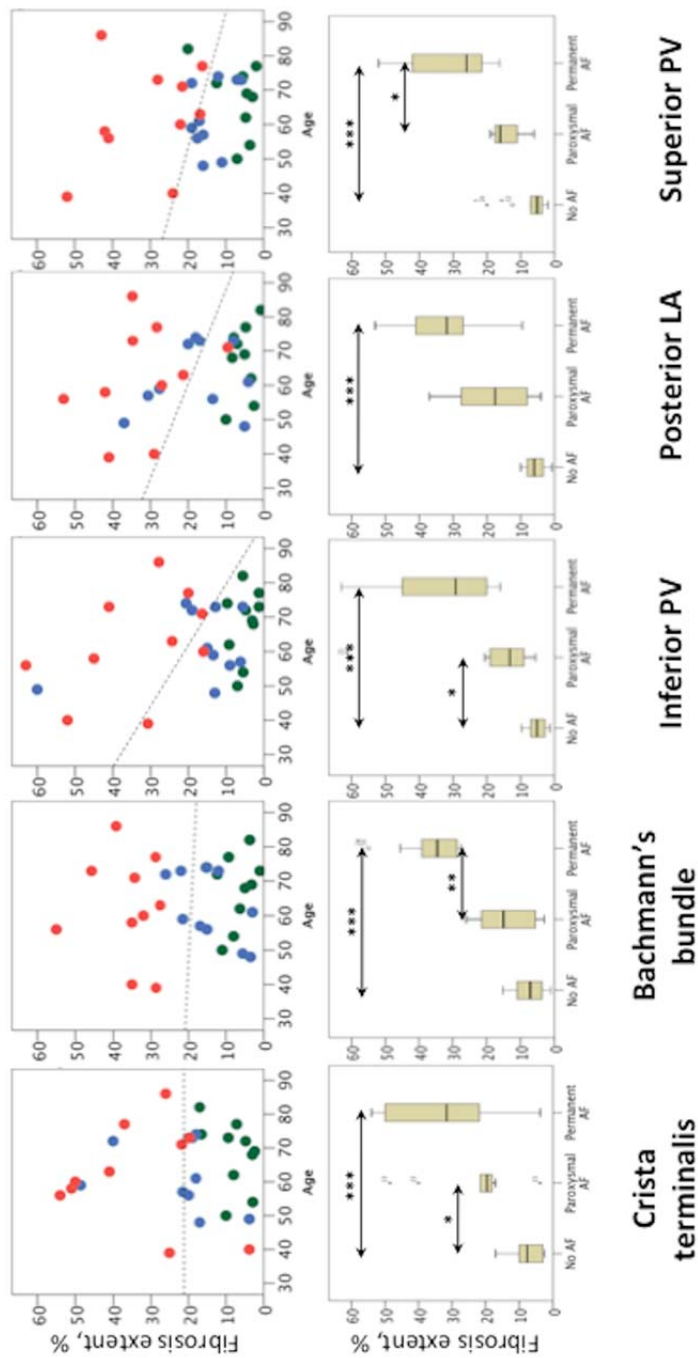


Figure 3: Fibrosis extent, age and clinical type of atrial fibrillation

Association between fibrosis extent, age (upper panels) and atrial fibrillation 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. AF= atrial fibrillation, LA= left atrium, PV= pulmonary vein. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001

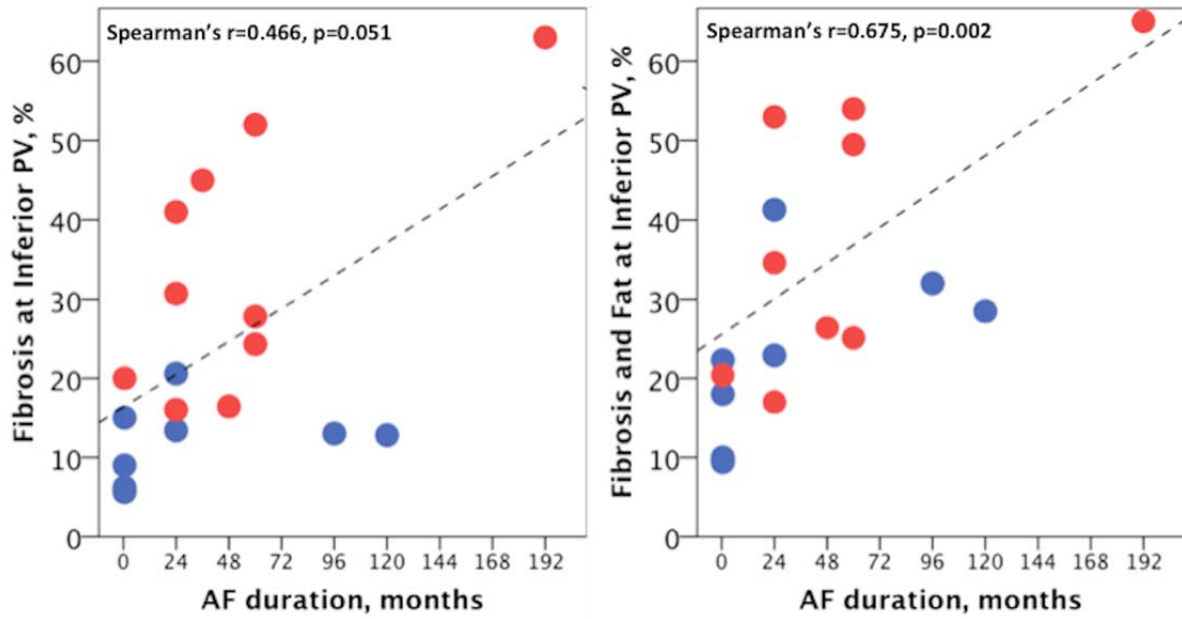


Figure 4: Association between fibrosis, fat and duration of AF history

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

Table 1: Patient characteristics

	Atrial fibrillation history		
	No AF n=10	Paroxysmal AF n=10	Permanent AF n=10
<b>Age, years</b>	68±10	62±10	61±15
<b>Male, n</b>	8	4	5
<b>BMI</b>	26,4±7,1	28,7±6,8	23,4±6,8
<b>Leftatrial diameter, mm</b>	40± 5	48± 8	56±13*
<b>LVEF, %</b>	39±15	40±19	41± 7
<b>QRS≥120 ms</b>	3	5	1
<b>Comorbidities:</b>			
Ischemic heart disease, n	10	7	9
Congestive heart failure, n	10	9	10
Hypertension, n	8	9	8
Diabetes mellitus, n	3	1	4
Stroke, n	3	2	3
COPD, n	4	2	0
CHA <sub>2</sub> DS <sub>2</sub> -VASc score	4,3±1,9	3,3±1,5	4,3±1,9
CHADS <sub>2</sub> -score	2,8±1,2	2,4±1,2	2,9±1,4
<b>Pharmacologicaltherapy:</b>			
ACE inhibitors or AT-2 receptor blockers, n	10	8	10
Statins, n	6	3	3
Amiodarone, n	0	3	3
Beta-blockers, n	8	9	10
Digoxin, n	1	0	6**

\*p=0.026 in comparison with NoAF group; \*\*p=0.004 in comparison with NoAF and PxAF groups

ACE= angiotensin-converting enzyme; AT= angiotensin; AF= atrial fibrillation; BMI= body mass index; COPD= chronic obstructive pulmonary disease; LVEDD= left ventricular end-diastolic diameter; LVEF= left ventricular ejection fraction;



Table 2: Fibrosis and fatty tissues in relation to age and AF history

	Age		Atrial fibrillation history	
	< 60 years n=11	≥ 60 years n=19	No AF n=10	AF n=20
<b>Fibrosis, %</b>				
Crista terminalis	23±19	20±14	<b>8±5</b>	<b>28±15***</b>
Bachmann's bundle	21±16	18±14	<b>8±4</b>	<b>25±14***</b>
Inferior PV	28±23	13±10	<b>5±3</b>	<b>26±18***</b>
Posterior LA	<b>26±17</b>	<b>14±11*</b>	<b>6±3</b>	<b>25±13***</b>
Superior PV	23±16	14±10	<b>7±5</b>	<b>22±13***</b>
<b>Fatty tissue, %</b>				
Crista terminalis	8±7	9±10	<b>3±5</b>	<b>12±10**</b>
Bachmann's bundle	13±13	7±4	<b>4±4</b>	<b>13±10**</b>
Inferior PV	10±11	7±7	<b>4±4</b>	<b>8±5*</b>
Posterior LA	7±5	6±5	<b>3±3</b>	<b>8±5**</b>
Superior PV	9±11	9±9	<b>4±3</b>	<b>12±10**</b>
<b>Fibrosis and Fatty tissue, %</b>				
Crista terminalis	32±22	29±18	<b>12±7</b>	<b>40±17***</b>
Bachmann's bundle	34±19	26±17	<b>11±5</b>	<b>38±16***</b>
Inferior PV	37±26	20±15	<b>9±5</b>	<b>35±20***</b>
Posterior LA	33±20	20±13	<b>8±4</b>	<b>33±15***</b>
Superior PV	32±18	23±15	<b>11±4</b>	<b>34±15***</b>
<b>Capillary density, %</b>				
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
<b>Cardiomyocyte diameter, μm</b>				
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

LA= left atrium, PV= pulmonary vein.

\* - p<0.05; \*\* - p<0.01; \*\*\*p<0.001



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
<b>CD45+ cell count</b>				
- 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***
<b>CD3+ cell count</b>				
- 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***

\* - p<0.05; \*\* - p<0.01; \*\*\*p<0.001 in comparison with No AF Group