Determinants and consequences of atrial fibrosis in patients undergoing open heart surgery

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Abstract

Objective: Atrial fibrillation (AF) is a frequent complication following open-heart surgery (OHS). Increased atrial fibrosis may indicate the presence of an intrinsic arrhythmogenic substrate. The aim of this prospective study was to determine whether atrial fibrosis is associated with increased prevalence of AF after OHS. Methods: Right atrial appendages were obtained from 259 patients undergoing OHS; none of the patients had a history of AF. Atrial fibrosis was quantitatively analyzed with point counting. All patients were followed prospectively until hospital discharge. None of the patients received anti-arrhythmic prophylaxis. Post-operative AF was defined as an episode of AF lasting $\geq 5$ min. Results: Quantitation of atrial fibrosis yielded a mean volume percentage of $15.8 \pm 4.3\%$ (V%; range 4.6–32.4%). Patient age was found to correlate with the amount of atrial fibrosis ($r=0.165; P<0.01$) and surface P-wave duration ($r=0.249; P<0.01$). The degree of fibrosis combined with P-wave duration predicted post-operative AF ($P<0.01$). Age (>60 years) and P-wave duration ($\geq 100$ ms) were independent predictors of post-operative AF (age: relative risk 2.20; P-wave: relative risk 2.69; $P<0.05$). The patients were divided into three groups: group 1, $V\% = 4.6–13.8\%$; group 2, $V\% = 13.9–23.1\%$; group 3, $V\% = 23.2–32.4\%$. A total of 52 patients (20.1%) developed AF, which occurred least commonly in group 1 (16.3%) and group 2 (21.2%) as compared with group 3 (33.3%). Conclusions: Atrial fibrosis provides a pathophysiological substrate for post-operative AF. The results support the importance of P-wave duration as a predictor of post-operative AF, and explain the increased prevalence of AF in elderly patients after OHS. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Arrhythmia (mechanisms); Atrial function; Cardiovascular surgery; Fibrosis; Supraventricular arrhythmia

1. Introduction

Atrial fibrillation (AF) is a frequent complication follow-
the pathophysiological mechanisms by which age or pre-existing cardiac abnormalities influence the occurrence of AF after cardiac surgery are unclear.

Animal studies have shown that atrial fibrosis can provide a morphological substrate for AF [8]. In addition, morphological studies suggest that the amount of fibrotic tissue is increased in patients with AF [9–11]. Thus, increased amounts of collagen deposits in the atria may increase the likelihood of AF after OHS. To date, the impact of pre-existing atrial histological abnormalities on post-operative AF has not been validated.

Therefore, in this prospective study, we investigated right atrial appendages for a correlation between the amount of atrial fibrosis and the prevalence of AF after OHS. To the best of our knowledge, this is the first large-scale prospective study investigating the potential contribution of atrial fibrosis in relation to already reported factors, such as atrial conducting abnormalities, on the occurrence of post-operative AF in patients undergoing OHS.

### Methods

#### 2.1. Patient population

Right atrial appendages were obtained from 259 consecutive patients undergoing cardiac by-pass surgery, mitral/aortic valve replacement and other surgical procedures (i.e. atrial septum defect (2 patients), resection of cardiac aneurysm (2), pericardiotomy and pericardiectomy (1), lobectomy of the lung (1)) at the Department of Cardiothoracic Surgery, University Hospital Magdeburg, Germany (Table 1). All surgical procedures were performed between October 20th 1999 and March 15th 2000. To be included in the study, patients had to be over 18 years and scheduled for elective OHS requiring cardiopulmonary by-pass. Patients were excluded if they had any of the following: a history of atrial fibrillation, atrial flutter or atrial tachycardias; administration of anti-arrhythmic drugs other than beta-receptor antagonists or calcium antagonists; participation in another protocol; uncontrolled renal, NYHA denotes New York Heart Association, ACE angiotensin converting enzyme, AT1 angiotensin 1, CABG coronary-artery by-pass grafting, AVR aortic valve replacement, and MVR mitral valve replacement, n.d. indicates not determined.

#### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total n=259</th>
<th>No AF after surgery n=207</th>
<th>AF after surgery n=52</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>63±10</td>
<td>62±11</td>
<td>66±7</td>
<td>0.011</td>
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<tr>
<td>Gender (m/f)</td>
<td>192/67</td>
<td>152/55</td>
<td>40/12</td>
<td>0.724</td>
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<tr>
<td>Diabetes mellitus [no. (%)]</td>
<td>86 (33)</td>
<td>65 (31)</td>
<td>21 (40)</td>
<td>0.453</td>
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<tr>
<td>Hyperlipoproteinemia [no. (%)]</td>
<td>233 (90)</td>
<td>185 (89)</td>
<td>48 (92)</td>
<td>0.796</td>
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<tr>
<td>Hypertension [no. (%)]</td>
<td>218 (84)</td>
<td>172 (83)</td>
<td>46 (89)</td>
<td>0.402</td>
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<tr>
<td>Three vessel coronary artery disease [no. (%)]</td>
<td>177 (68)</td>
<td>141 (68)</td>
<td>36 (69)</td>
<td>0.755</td>
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<tr>
<td>Myocardial infarction [no. (%)]</td>
<td>107 (41)</td>
<td>84 (41)</td>
<td>23 (44)</td>
<td>0.141</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>2.1±1.1</td>
<td>2.1±1.1</td>
<td>2.2±1.1</td>
<td>0.875</td>
</tr>
<tr>
<td>Ejection fraction [%]</td>
<td>57.1±13.9</td>
<td>57.1±13.8</td>
<td>57.3±14.6</td>
<td>0.907</td>
</tr>
<tr>
<td>P-wave width (ms)</td>
<td>91±17</td>
<td>81±12</td>
<td>102±16</td>
<td>0.010</td>
</tr>
<tr>
<td>Creatinine before surgery (µmol/l)</td>
<td>86±36.5</td>
<td>87±39.5</td>
<td>84±20.8</td>
<td>0.663</td>
</tr>
<tr>
<td>C-reactive peptide before surgery (mg/l)</td>
<td>15.0±28.6</td>
<td>15.1±29.2</td>
<td>14.7±26.3</td>
<td>0.942</td>
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<tr>
<td>C-reactive peptide after surgery (mg/l)</td>
<td>65.3±47.1</td>
<td>64.0±44.0</td>
<td>69.9±57.5</td>
<td>0.424</td>
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<tr>
<th>Therapy before heart surgery:</th>
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</thead>
<tbody>
<tr>
<td>Beta-blocker [no. (%)]</td>
<td>112 (43)</td>
<td>91 (44)</td>
<td>21 (40)</td>
<td>0.754</td>
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<tr>
<td>Digitalis [no. (%)]</td>
<td>34 (13)</td>
<td>24 (12)</td>
<td>10 (19)</td>
<td>0.168</td>
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<tr>
<td>Calcium-channel blocker [no. (%)]</td>
<td>26 (10)</td>
<td>16 (7)</td>
<td>10 (19)</td>
<td>0.020</td>
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<tr>
<td>ACE-inhibitor/AT1-antagonists [no. (%)]</td>
<td>156 (60)</td>
<td>124 (59)</td>
<td>32 (62)</td>
<td>0.875</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of heart surgery:</th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>CABG [no. (%)]</td>
<td>194 (74)</td>
<td>155 (74)</td>
<td>39 (75)</td>
<td>0.986</td>
</tr>
<tr>
<td>CABG+valve replacement [no. (%)]</td>
<td>30 (11)</td>
<td>23 (11)</td>
<td>7 (14)</td>
<td>0.636</td>
</tr>
<tr>
<td>Valve replacement [no. (%)]</td>
<td>29 (11)</td>
<td>24 (12)</td>
<td>5 (10)</td>
<td>0.686</td>
</tr>
<tr>
<td>AVR [no. (%)]</td>
<td>24 (9)</td>
<td>22 (11)</td>
<td>2 (4)</td>
<td>0.132</td>
</tr>
<tr>
<td>MVR [no. (%)]</td>
<td>3 (1)</td>
<td>0 (0)</td>
<td>3 (6)</td>
<td>n.d.</td>
</tr>
<tr>
<td>AVR+MVR [no. (%)]</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Other [no. (%)]</td>
<td>6 (2)</td>
<td>5 (2)</td>
<td>1 (2)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Aorta cross-clamp time (min)</td>
<td>62±23</td>
<td>62±24</td>
<td>61±20</td>
<td>0.886</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>198±47</td>
<td>198±48</td>
<td>201±44</td>
<td>0.600</td>
</tr>
<tr>
<td>Stimulation after surgery [no. (%)]</td>
<td>92 (36)</td>
<td>72 (35)</td>
<td>20 (39)</td>
<td>0.630</td>
</tr>
</tbody>
</table>
hepatic, or heart failure. All patients gave written consent to participating in the study and their baseline characteristics are shown in Table 1. The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. Study protocol

All patients were hospitalized 1 day before surgery. After a clinical examination, a 12-lead ECG was recorded for each patient, and routine blood samples were taken to determine white and red blood cell counts, hepatic and renal function, and the C-reactive protein level. All surgical procedures were performed using extracorporeal circulation. During surgery, myocardial protection was provided by cold cardioplegia (Bretschneider solution) in every patient. After the procedure, all patients were transferred to an intensive care unit where they were monitored continuously for at least 72 h. A 12-lead ECG was recorded daily and in the case of suspected arrhythmias during the period of hospitalization. P-wave duration was assessed preoperatively using measurements from two independent observers in lead II from a standard 12 lead surface ECG (50 mm/s paper speed). AF episodes lasting longer than 5 min were counted as an episode of post-operative AF and treatment depended on the clinical presentation of the patient. Pre-operative medication with beta-blockers was continued after surgery. The remaining patients received no specific post-operative medical prophylaxis for AF. All patients were discharged from hospital approximately 11 days after surgery.

2.3. Quantitation of atrial fibrosis

Tissue samples (one or two entire cross sections, depending on the amount of tissue available) of the right atrial appendages were fixed in formalin and embedded in paraffin. Deparaffinized sections were stained with hematoxylin and eosin and van Gieson’s elastic stain (EvG). Van Gieson’s elastic stain allows for the differentiation between elastin (black staining) and collagen (bright red staining). The quality of staining was assessed by two independent observers. Atrial fibrosis, including interstitial and perivascular fibrosis, was quantitatively analyzed with point counting on EvG-stained paraffin sections (Fig. 1). A 144-point multipurpose microgrid was fitted in a 12.5× ocular of a microscope equipped with a 16× objective, yielding a test field size of 0.289 mm² at the specimen level. Points falling on red-stained, collagen-rich fibrotic areas were counted in 15 randomly selected fields per-specimen. The volume percentage (V%) of fibrosis was estimated using the mean number of points-per-field, in which 144 points-per-field were equal to 100%. The pathologist investigating the biopsy specimens and quantitating the amount of fibrosis was unaware of the personal and clinical data of the patients.

2.4. Statistics

All values were expressed as mean±standard deviation (S.D.). Continuous variables were compared by the unpaired Student’s t-test. The \( \chi^2 \)-test, multivariance analysis,
and the principal-component-multivariance method [12,13] were used, where appropriate, to assess the association between post-operative AF and clinical parameters. The Pearson correlation coefficient was used to determine the relationship between metric parameters. A value of $P<0.05$ was considered to be statistically significant.

### 3. Results

#### 3.1. Patient characteristics

The clinical characteristics of the patients are summarized in Table 1.

#### 3.2. Atrial fibrosis

Atrial fibrosis was characterized by progressive thickening of the interstitial matrix initially involving groups of myocytes (Fig. 1A), subsequent separation of individual myocytes (Fig. 1B), and, finally, formation of large patches of confluent fibrotic areas (Fig. 1C). The volume percentage of atrial fibrosis, as quantified by point counting, ranged from 4.6 to 32.4% (15.8±4.3%). The amount of right atrial fibrosis was found to be similar in patients with various heart diseases (e.g. coronary artery disease vs. valve diseases or septal defects). There was a linear relationship between patient age and amount of fibrosis ($r=0.17; P<0.01$; Fig. 2). The amount of right atrial fibrosis did not correlate with left ventricular ejection fraction, atrial diameter, or with other metric clinical parameters (data not shown).

#### 3.3. Post-operative AF

A total of 52 (20.1%) of the 259 patients studied developed AF within 72 h after cardiac surgery. The clinical characteristics (gender, left ventricular ejection fraction, atrial size, underlying cardiac disease, perioperative parameters, post-operative use of beta-blockers) were comparable in patients both with and without post-operative AF (Table 1) except for calcium channel blockers (Table 1). However, on multivariance analysis, the use of calcium-channel blockers ($n=26$) was no independent predictor of AF. A significant association ($\chi^2=6.908; P<0.05$) was found between patient age and the occurrence of post-operative AF (Fig. 3); the incidence of AF was 6.3% in patients aged 45 years and younger ($n=16$), and 12.3% in patients aged between 45 and 60 years ($n=73$). The highest incidence of post-operative AF (24.7%) was found in patients over 60 years ($n=170$; Fig. 3). The relative risk of post-operative AF was 2.20 times higher (95% CI: 1.21–4.03) in those above 60 years of age compared with those below 60 years of age (Table 1). A calculated score on multivariance analysis [11,12] including age and fibrotic content showed a significant association between these variables and post-operative AF (AF: 102±15 vs. 95±15; $P<0.01$).

Patient age correlated positively with surface $P$-wave duration ($r=0.249; P<0.01$). Similar to age, surface $P$-wave duration also predicted post-operative AF ($\chi^2=47.49; P<0.05$). A $P$-wave measuring $\geq 100$ ms was associated with a risk of AF that was 2.69 times higher (95% CI: 1.83–3.96) than for $P$-waves measuring $<100$ ms. $P$-wave duration did not correlate with the left atrial diameter ($P=0.721$). Similar to the observed association between age, fibrosis and AF, the calculated score on multivariance analysis that included $P$-wave duration and fibrotic content was significantly higher in patients with post-operative AF than in those without AF (AF: 92±15 vs. 72±10; $P<0.01$). Thus, a combination between the degree of fibrosis and $P$-wave duration yielded a signifi-
cant predictive value of post-operative AF (\(P<0.01\)). In multivariance analysis no other clinical parameter, including different types of heart diseases (valvular disease or septal defects), was an independent predictor of AF.

To assess a putative direct correlation between fibrosis and AF, the amount of fibrosis (spanning 4.6–32.4%) was divided into three categories, each spanning an equal range (9.2%) of fibrosis, i.e. 4.6–13.8, 13.9–23.1 and 23.2–32.4%. Following this categorization a linear relationship was found between the amount of atrial fibrosis and post-operative AF: the incidence of AF was 16.1% in patients with a fibrotic content between 4.6 and 13.8% (\(n=98\)), 21.2% in patients with a fibrotic content between 13.9 and 23.1% (\(n=146\)), and 33.3% in patients with 23.2–32.4% (\(n=15\)) fibrosis (Fig. 4).

4. Discussion

To the best of our knowledge, this is the first prospective study determining the relationship between a histological alteration, i.e. the amount of atrial fibrosis, and the occurrence of atrial fibrillation after OHS in a large cohort of patients. The amount of fibrosis in right atrial appendages, as shown here, varies considerably ranging from 4% to almost 33% of the tissue volume, and it may be of diverse etiology, including cardiac inflammation, ischemia, activation of the cardiac angiotensin system and aging [9,11,14–16]. In our series, the amount of atrial fibrosis correlated primarily with patient age, while other etiologies (e.g. coronary artery disease, valvular diseases) may have contributed to its development, these proved to be in-

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Fig. 3. Association between patient age and incidence of post-operative atrial fibrillation. *\(P=0.032\) vs. <45 years.

Fig. 4. Association between degree of atrial fibrosis (V%) and incidence of post-operative atrial fibrillation.
significant. Atrial fibrosis may not only indicate a disease process, it may also be a risk factor for the development of a cardiac disease: in our series patient age correlated with P-wave duration, and the amount of atrial fibrosis in association with prolongation of the surface P-wave or advanced age correlated with post-operative AF. These observations support the significance of age-related degenerative atrial changes (e.g. collagen accumulation) as the putative cause for slow conduction/conduction inhomogeneities and AF [8,17–19]. No association was found between left ventricular ejection fraction or underlying heart disease and the amount of atrial fibrosis, which keeps with previous findings [14,17]. A prolonged pre-operative P-wave duration predicts AF after OHS [3,5,20] and, as demonstrated in this study, a prolonged P-wave may be related to the presence of atrial fibrosis. Atrial fibrosis isolates groups of atrial myocytes and individual myocytes (Fig. 1). It impairs cell-to-cell coupling, thus causing inhomogeneities in intra- and interatrial conduction [9,21]. Initially, subsequent changes in atrial conduction may be subtle, and prolonged P-wave duration may be detectable only by signal-averaging techniques [3,5]. However, previous studies have shown that atrial fibrosis may provide a structural substrate for AF: areas of fibrotic tissue are included in macro re-entry circuits during AF [8], increased amounts of atrial fibrosis were found in patients with AF [9–11], and the amount of atrial fibrosis increases with patient age and duration of AF in chronic AF [22]. Our study, however, is the first to demonstrate an association between age-dependent histological alterations of atrial tissue from patients with sinus rhythm without a history of AF, surface P-wave duration, and the incidence of post-operative AF after OHS.

The molecular mechanisms responsible for age-related cardiac changes have not been fully elucidated. The aging heart loses about 38 million cardiomyocyte nuclei per-year [23], which may lead to replacement fibrosis, as was found in the aging hearts of Fischer rats [24]. In addition to replacement fibrosis, the presence of reactive cardiac fibrosis in the elderly, possibly induced by increased levels of transforming growth factor-beta, has been postulated [25,26]. Other profibrotic molecular mechanisms (angiotensin II, bradykinin, endothelin I, and others) influencing this process still need to be clarified.

Although we found a significant correlation between age, post-operative AF and atrial fibrosis, the amount of atrial fibrosis in patients with and without post-operative AF over-lapped markedly. Therefore, additional factors, such as electrophysiological mechanisms, appear to influence post-operative AF in the elderly. Michelucci et al. [19] suggested that aging modifies atrial refractoriness. They found a direct correlation between age and atrial effective refractory period (ERP) in the high right atrium, but not at other right atrial sites. This resulted in an age-dependent non-uniform, progressive increment of dispersion of atrial refractoriness, which is known to increase the risk of AF [27,28,]. It has recently been shown that gender influences the relationship between age and atrial ERP [29]. The age-related prolongation of the atrial ERP appears to be due to alterations of calcium and potassium currents in atrial myocytes [30]. In addition to ERP changes, altered anisotropic properties of atrial pectinate muscle bundles may further increase the likelihood for AF in elderly patients. Premature action potentials produce either an unidirectional longitudinal conduction block or a dissociated zigzag type of longitudinal conduction, allowing re-entry in very small areas (1–2 mm) [31]. This was not observed in muscle preparations from younger individuals [31]. Age-independent factors, such as atrial premature beats, catecholamines, electrolyte disturbances and inflammatory reactions, may also influence post-operative AF [1,2,32,33], which points to a multifactorial pathogenesis of post-operative AF. The relatively low r-values (which proved to be highly significant) observed in our series imply that other factors contribute to post-operative AF. Further studies are necessary to prove a putative amplifying effect of age-dependent and age-independent structural and electrophysiological alterations on post-operative AF.

In contrast to previous studies [1,34], the pre-operative use of calcium channel blockers in our study was found to be more common in patients with AF than in those without. However, the number of patients receiving Class IV drugs was limited (n = 26) and the use of calcium channel blockers was not an independent predictor of AF in multivarience analysis. A recently published prospective study of 330 patients has shown that calcium channel antagonists do not increase the incidence of post-operative AF [35], and therefore, it seems unlikely that the use of calcium channel blockers had a significant impact on the results of our study.

As left atrial appendages were not analyzed, no comment can be made about the interatrial distribution of atrial fibrosis, which may influence post-operative AF. In fact, it is necessary to sample larger amounts of human atrial tissue to answer these questions. However, this procedure is not feasible. To demonstrate a statistically significant association between age, P-wave duration and the risk of AF after OHS it presumably suffices to characterize right atrial fibrosis. The results of this study underline the putative role of atrial fibrosis as an ‘arrhythmogenic substrate’ for AF, which may provide a novel therapeutic target in patients with AF [11,18,36]. Future studies will have to prove, whether age-dependent atrial fibrosis responds to medical treatment, thus decreasing the incidence of AF in the elderly.

References


