Morphology of inter-atrial conduction routes in patients with atrial fibrillation

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Background Inter-atrial conduction disturbance is associated with the presence of atrial fibrillation (AF), although little is known about the anatomy of the inter-atrial connections.

Methods and Results Twenty-seven hearts from in-hospital deaths were examined and stratified in two groups with regard to their history of AF. Measurements of atrial weight were performed after excising the atria at the level of the atrioventricular valve plane and separation from the inter-atrial septum (IAS). In addition, in 15 of 27 hearts (seven AF, eight non-AF) the IAS was sliced into 10 μm thick parallel histological sections at intervals of 1 mm starting at the valve plane and ending at the atrial roof. The sections were stained with van Gieson’s stain. The variable morphology of the anterior and posterior inter-atrial connective muscle bundles is described. The total number of inter-atrial connections varied from 1 to 5. The anterior route (Bachmann’s bundle) was not found in seven of 15 specimens.

Conclusion Inter-atrial connections are characterized by substantial variability in morphology and in the distribution of bundles. This may account for part of the variable susceptibility to AF.

Introduction

Inter-atrial conduction disturbances are frequently associated with the presence of atrial fibrillation (AF) [1,2]. However, being based on animal studies [3] and anatomical studies in humans [4–8], our knowledge of the exact locations of inter-atrial routes is still incomplete. Recent advances in the non-pharmacological management of AF, such as chronic multi-site or single site inter-atrial septal pacing [9,10], as well as atrial compartmentalization procedures achieved either surgically [11] or using the radiofrequency catheter ablation technique [12] have increased interest in the exact anatomy of the inter-atrial connections. We have earlier shown that the configuration of the signal-averaged P-wave ECG in patients with lone AF suggests the presence of conduction delay in the posterior inter-atrial septal region [13]. This association of inter-atrial conduction disturbance in the postero-septal region with AF received further support from a series of endocardial electrophysiological studies performed in patients with lone AF [4,15]. The present autopsy study was designed to investigate the possible macro- and microscopic differences of inter-atrial connections and inter-atrial septal morphology in patients with and without a previous history of AF.

Methods

Post-mortem studies were performed in 27 patients (69 ± 10 years, range 50–90 years, 14 females) in St Petersburg (Russia) as part of normal hospital routine. Twenty-two patients died from complications of coronary artery disease, two from pneumonia, two from stroke, and one from bleeding. Group 1 (AF) was composed of 12 patients (69 ± 7 years, 7 females) with AF (chronic in nine and paroxysmal in three). The other 15 patients (68 ± 12 years, 7 females) did not show any evidence of AF in their medical documentation (Group 2, non-AF). In addition, macroscopic evaluation was made of 11 hearts from normal individuals without known chronic disease who either committed suicide or
died accidentally (mean age 35 ± 14 years, range 20–56 years, two females) — Group 3 (Control). There were no significant differences in age between Groups 1 and 2. The ages in Group 3 were significantly lower than those in the other two groups (P<0·0001 for both comparisons).

During the initial stage of the study, manual preparation and epicardial fat removal from the posterior inter-atrial groove was performed on the first seven hearts in an attempt to visualize the posterior inter-atrial connections. However, in order to avoid potential damage to any tiny structures located in the groove, this was not done later.

Macroscopic evaluation followed a standard autopsy protocol. Atria were excised at the level of the valve plane and separated from the inter-atrial septum so as to leave 3–5 mm of the atrial walls both anteriorly and posteriorly (Fig. 1). The separated parts of the right and the left atria were used for atrial mass measurements. The dimensions of the inter-atrial septum and its components were also assessed. The narrowest widths of the muscular rim surrounding the fossa ovalis were measured anteriorly and posteriorly in order to assess the ‘isthmus widths’. The area of the fossa ovalis was estimated as the product of its length and width.

In 15 patients (seven AF and eight non-AF), light microscopy of the histological layers of the inter-atrial septum was performed. The inter-atrial septum specimens were fixed in paraffin and then sliced into the consecutive layers 10 μm thick with a microtome. The layers were made parallel to the valve plane between the valve plane and the atrial roof in 1-mm steps. All layers were then stained with van Gieson’s stain. The number of layers varied from 30 to 60 per heart.

‘Inter-atrial’ bundles were defined as muscle fibres, which crossed the microscopic slide transverse to the axis of the inter-atrial septum and parallel to the section plane. The width of each bundle was calculated from the number of consecutive parallel slides with a known distance (1 mm) between them in which the fibres were present. The thickness of the bundle was taken as its maximal thickness in the histological slide. The cross sectional area of the bundle was defined as the product of the width and thickness.

The results were analyzed using the non-parametric Mann–Whitney U-test for unpaired variables. All results are expressed as means ± standard deviations. Statistical significance was indicated by a P-value<0·05.

Results

Macroscopic evaluation

The results of the organometric measurements are presented in Table 1. Although no significant differences in

Table 1 Macroscopic evaluation of the study material

<table>
<thead>
<tr>
<th>Variable</th>
<th>AF Group (1)</th>
<th>Control Group (3)</th>
<th>Non-AF Group (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=12</td>
<td>n=15</td>
<td>n=11</td>
</tr>
<tr>
<td>LA mass [g]</td>
<td>31 ± 12</td>
<td>13 ± 7</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>RA mass [g]</td>
<td>36 ± 13</td>
<td>15 ± 5</td>
<td>30 ± 8</td>
</tr>
<tr>
<td>IAS mass [g]</td>
<td>11 ± 4</td>
<td>4 ± 2</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>FO area [cm²]</td>
<td>3 ± 1</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Anterior isthmus [mm]</td>
<td>11 ± 11</td>
<td>6 ± 3</td>
<td>15 ± 9</td>
</tr>
<tr>
<td>Posterior isthmus [mm]</td>
<td>9 ± 8</td>
<td>7 ± 5</td>
<td>10 ± 5</td>
</tr>
</tbody>
</table>

LA=left atrium; RA=right atrium; IAS=inter-atrial septum; FO=fossa ovalis.

Differences of the macroscopic parameters between Groups 1 and 2 are not significant.
organometric parameters were found between the groups, the right and left atrial masses were higher in the AF than in the non-AF group. On the other hand, the left and right atrial masses of the hearts of patients who died from ischaemic heart disease were significantly greater than in normals (Group 3). No significant differences in the inter-atrial septum area were observed.

**Inter-atrial connections, macroscopic study**

Manual removal of epicardial fat from the posterior inter-atrial groove in normal hearts revealed different kinds of inter-atrial bundles connecting the atria. These varied from several thin (about 1 mm thick) fibres adjacent to the atrial walls to distinct bundles of fibres crossing the groove in the epicardial fat above the coronary sinus or attached to the atrial walls (Fig. 2). The technique did not permit identification of the presence of blood vessels supplying the bundles. Light microscopy of histological sections of the bundles confirmed they consisted of working myocytes.

**Inter-atrial connections, microscopic study**

Evaluation of the inter-atrial connections with light microscopy revealed variability in the distribution of the bundles (Table 2). The anterior bundle (known as Bachmann’s bundle) was present in only seven of the 15 hearts studied with serial histological sections. In the other eight hearts, no signs of transverse bundles between anterior isthmus and posterior segment of the aortic wall were seen. In two of the hearts with obvious Bachmann’s bundles, a muscle bundle coming from the inter-atrial septum and converging with Bachmann’s bundle at the anterior left atrial wall was found (Fig. 3). In one patient, the anterior bundle was split into two layers separated by fatty tissue. The maximum cross sectional area of the bundles was 18 \( \times \) 3 mm\(^2\). In three of these hearts, no posterior connection could be found, i.e. Bachmann’s bundle was the only inter-atrial route in these hearts. In the other patients, a variety of muscle fibres connected the atria posteriorly (Fig. 4). The inter-atrial connections could be described as anterior (three hearts), posterior (eight hearts) or of mixed type (four hearts). The last is characterized by a combination of an apparent Bachmann’s bundle and posterior connections. The mean number of connective bundles was 2.0 \( \pm \) 1.8 in the AF group and 2.6 \( \pm \) 1.2 in the non-AF group (NS).

The posteriorly located muscle bundles could be classified into two groups based on their relation to the atrial wall: (a) a ‘path’ lying entirely beneath the epicardial fat adjacent to the atrial walls and merging with the atrial wall muscle fascicles and (b) a ‘bridge’ connecting two sites on the posterior atrial walls (i.e. one on the right side with another one on the left side of the inter-atrial groove) with the space filled by epicardial fat between the bundle and the atrial walls.

The bundles were located posterior to the coronary sinus (four hearts), between the coronary sinus and the posterior isthmus of the inter-atrial septum (four hearts), and above the coronary sinus (seven hearts). In three cases, the bundles were parts of the inter-atrial septum located within the posterior isthmus and separated from the vertically oriented muscle fibres. Blood vessels were always observed in the vicinities of the bundles.

The numbers and dimensions of the posterior connections varied substantially between the hearts, from a distinct single path-type bundle of 22 \( \times \) 1 mm\(^2\) cross sectional area (in a patient without a Bachmann’s bundle) to five separate bundles lying in the epicardial fat at different levels above the coronary sinus. Bridge-type connections were observed in 11 of the 12 hearts in which posterior bundles were present. This was the only type of posterior connection in eight of these 12 hearts. In three patients, both types of posterior connections were present. One heart had only one posterior bundle adjacent to the atrial wall. No correlations between the types and/or numbers of posterior connections and AF history were observed.

**Discussion**

**Previous studies**

The role of inter-atrial conduction in the development of AF is incompletely explored. While prolongation of inter-atrial conduction is a frequent finding in patients with paroxysmal and chronic AF\([16–18]\) complete abolition of inter-atrial conduction by atrial compartmentalization procedures was suggested to eliminate arrhythmia\([11,19]\). On the other hand, there are studies which report significant conduction delays within the right atrium, specifically related to delays in activating the coronary sinus ostium, thereby causing overall prolongation of inter-atrial conduction\([14,20]\).

Present understanding of inter-atrial conduction routes is based on limited anatomical studies in humans\([4–7]\). Based on electroanatomical mapping of the atria in patients without structural heart disease, recent reports suggest two major inter-atrial activation routes\([21,22]\). The anterior inter-atrial band described by Bachmann\([23]\) is a well-recognized circumferential muscle bundle located in the anterior wall of the left atrium and connecting the right and left atrial appendages. On the other hand, little is known about the posterior inter-atrial route. Chauvin et al\([8]\) have found consistent but variable connections between the coronary sinus musculature and the left atrium. Although inter-atrial conduction via coronary sinus tissues has also been verified in an experimental study\([24]\), this may not be limited to the two routes, as shown by Roithinger.
Figure 2. Different types of posterior inter-atrial connections. View of the posterior inter-atrial groove with the epicardial fat removed. (A) Multiple inter-atrial bundles; (B) single inter-atrial connective bundle crossing the posterior inter-atrial groove in the epicardial fat (‘bridge’ type connection). The inset shows the space between the posterior bundle and the atrial wall. The broken line indicates the projection of the posterior inter-atrial groove; (C) single inter-atrial connective bundle attached to the atrial walls (‘path’ type connection). LA=left atrium; RA=right atrium; CS=coronary sinus.
who reported a left-to-right inter-atrial septal breakthrough, also occurring in the vicinity of the oval fossa.

Variability of inter-atrial activation routes

In view of previous studies, the results of our investigation show that inter-atrial routes are non-uniform structures subjected to substantial variability as regards location, dimensions, and distributions of their components. This is in agreement with a recently published paper of Ho and colleagues, who described variable connections between the right and left atria and demonstrated the presence of bundles extending into the pulmonary vein musculature[7]. However, the anterior route (such as via Bachmann’s bundle) recognized in earlier anatomical studies was not found in half of the hearts examined, in which the posterior type of inter-atrial connections was seen. That Bachmann’s bundle is not an ubiquitous structure has been reported earlier by Mikhailov and Chukbar[6] who examined 77 human hearts observing Bachmann’s bundle in only 55% of the histological sections, and in only 29% of the specimens macroscopically visible anterior bundles were seen. This observation was recently supported by use of a novel non-contact technique for left atrial mapping[25]. The study demonstrated that during sinus rhythm the left atrium may be activated mainly through posterior septal fibres thus suggesting that Bachmann’s bundle is less important than previously considered.

On the other hand, in our study, posterior connections were not observed in the three hearts with anterior inter-atrial connections. It is possible that in these cases connections between the coronary sinus musculature and the left atrial wall providing posterior inter-atrial conduction were present outside the line at which the inter-atrial septum was excised and therefore not seen in the microscopic slides. In fact, we were able to identify muscular fascicles passing through the inter-atrial septum and merging with the coronary sinus musculature (see Table 2) similar to the findings of connections between the coronary sinus and the left atrium reported earlier[8]. Exploring this in detail was however not, however, the purpose of the study. Variability in the sizes and locations of the conductive bundles suggests differences in predisposition to cardiac arrhythmia, due to the potentially increased vulnerability of the bundles lying separately within the fatty tissue. However, the design of the present study did not permit the demonstration of any such differences.

Clinical implications

Recent developments in the non-pharmacological management of AF are based on improved knowledge of the pathophysiological mechanisms of arrhythmia. Multi-

<table>
<thead>
<tr>
<th>Age [years]</th>
<th>Group</th>
<th>BB size [mm]</th>
<th>No. of PBs</th>
<th>PB type</th>
<th>PB location</th>
<th>Maximal PB size [mm]</th>
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<tbody>
<tr>
<td>69</td>
<td>AF</td>
<td>—</td>
<td>1</td>
<td>B</td>
<td>Anterior (+ branch to LA wall)</td>
<td>13 × 0.5</td>
</tr>
<tr>
<td>68</td>
<td>AF</td>
<td>5 × 10</td>
<td>0</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>61</td>
<td>AF</td>
<td>—</td>
<td>1</td>
<td>B</td>
<td>High (posterior isthmus)</td>
<td>3 × 0.3</td>
</tr>
<tr>
<td>70</td>
<td>AF</td>
<td>—</td>
<td>1</td>
<td>B</td>
<td>High</td>
<td>2 × 0.5</td>
</tr>
<tr>
<td>77</td>
<td>AF</td>
<td>18 × 3</td>
<td>5</td>
<td>B</td>
<td>High (4)</td>
<td>3 × 1</td>
</tr>
<tr>
<td>75</td>
<td>AF</td>
<td>—</td>
<td>3</td>
<td>B+P</td>
<td>Posterior (2)</td>
<td>11 × 3</td>
</tr>
<tr>
<td>67</td>
<td>AF</td>
<td>5 × 5</td>
<td>1</td>
<td>B</td>
<td>High</td>
<td>1 × 2</td>
</tr>
<tr>
<td>77</td>
<td>Non-AF</td>
<td>4 × 3*</td>
<td>—</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>80</td>
<td>Non-AF</td>
<td>6 × 4†</td>
<td>—</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>65</td>
<td>Non-AF</td>
<td>—</td>
<td>1</td>
<td>P</td>
<td>High</td>
<td>22 × 1</td>
</tr>
<tr>
<td>75</td>
<td>Non-AF</td>
<td>40 × 1†</td>
<td>3</td>
<td>B</td>
<td>Surrounding CS</td>
<td>34 × 2</td>
</tr>
<tr>
<td>61</td>
<td>Non-AF</td>
<td>7 × 2</td>
<td>2</td>
<td>B</td>
<td>Surrounding CS</td>
<td>2 × 0.8</td>
</tr>
<tr>
<td>65</td>
<td>Non-AF</td>
<td>—</td>
<td>2</td>
<td>B</td>
<td>Surrounding CS</td>
<td>8 × 0.3</td>
</tr>
<tr>
<td>50</td>
<td>Non-AF</td>
<td>—</td>
<td>3</td>
<td>B+P</td>
<td>High</td>
<td>2 × 1</td>
</tr>
<tr>
<td>90</td>
<td>Non-AF</td>
<td>—</td>
<td>2</td>
<td>B+P</td>
<td>High</td>
<td>6 × 0.5</td>
</tr>
</tbody>
</table>

*BB is split in two bands separated by fatty tissue.
†BB gives a branch to the inter-atrial septum.
BB=Bachmann’s bundle; PB=posterior inter-atrial bundle; AF=known atrial fibrillation (Group 1); Non-AF=no history of atrial fibrillation (Group 2); B=’bridge’ type of PB; P=’path’ type of PB; PB location=location of PB in relation to the CS.
or single-site inter-atrial septal pacing, either in the coronary sinus ostium region\(^{10}\) or at the putative insertion of Bachmann’s bundle\(^{26}\), has been introduced for preventing AF paroxysms. The mechanism reduces the global atrial activation time and eliminates the delay in inter-atrial conduction, thereby not favouring the occurrence of the re-entrant circuit and the subsequent development of AF. Initial experience of these pacing
Figure 4. Microphotograph of the posterior inter-atrial bundle (van Gieson’s staining). Fibrous tissue is stained red. Note the right-to-left orientation of the muscle bundles and fibrous matrix surrounding muscle fascicles in the bundle (× 100). The location of the enlarged segment is circled in the inter-atrial septum overview slide (left panel). Arrows indicate the posterior-anterior axes. LA=left atrium; RA=right atrium; CS=coronary sinus.
modalities shows promising results. Understanding of the variability of the inter-atrial connections is important for understanding the difficulties encountered during pacing. Theoretically, the optimal site for placing electrodes for septal pacing in the right atrium would be the site where the inter-atrial connective bundle crosses the inter-atrial septum. This would minimize delays in transseptal conduction and reduce the inter-atrial conduction times. However, no technique for verifying correct bundle insertion has been developed. The variability in its location discovered in the present study may affect the protective effects of septal pacing for AF.

The observation of tiny connective fibres in the epicardial fat of the posterior inter-atrial groove suggests increased potential vulnerability of these structures, possibly predisposing to AF. It might be suggested that more morphologically- and functionally-developed anterior and posterior inter-atrial connections provide more homogenous right-to-left activation spread, thereby reducing the probability of ‘triggering’ re-entry and vice versa. This was supported by findings using signal-averaged P-wave ECG in the AF patients compared with healthy subjects[13]. Atrial fibrillation patients showed P-wave configuration suggesting delayed conduction in the posterior septal region and retrograde activation of the coronary sinus. In a series of endocardi-
dial electrophysiological sessions, we have earlier shown that patients with lone AF show significant conduction delays, which may be a part of the AF initiation mechanism, in the vicinity of the coronary sinus ostium and the posterior septal regions[14].

In the present study, while the total numbers of connective bundles was smaller in the AF group than in patients without AF history, the differences were not statistically significant. It is not known whether these differences are individual variations or result from remodelling processes leading to the disappearance of functioning connections in the posterior inter-atrial groove. It is also not known to what extent the morphology of the inter-atrial connections affects inter-atrial conduction but the marked variability of morphology may suggest a variability of function, and therefore affect predisposition to AF.

Limitations of the study

One of the most important limitations in the technique used was that it only permitted connections whose axes corresponded to the section planes. If the muscle fibres crossed the planes of the slides at certain angles, they appear as cross rather than longitudinal sections in the slides and, hence, could not be described as ‘transseptal’ bundles. This could potentially under-estimate the actual numbers of connective bundles and account for the discrepancy with clinical studies, which consistently show the presence of two major connective routes. Though unlikely, if its orientation was far from the section plane this limitation might theoretically result in the ‘absence’ of Bachmann’s bundle. We were not able to verify the presence of macroscopically-visible inter-atrial connections in the specimens subjected to light microscopy of their serial sections.

Conclusion

The variability of the inter-atrial connective bundles seen in this study alters some of the current views on the anatomy of inter-atrial connections. The absence of the anterior Bachmann’s bundle in half the patients and potentially vulnerable posterior connections might explain why some patients are more prone to develop inter-atrial conduction blocks and AF than others. These findings show the need for more extensive studies in a larger material.

The authors thank Natasha Kazankova (Research Institute of Cardiology, St Petersburg) and Bibi Smideberg (Department of Cardiology, Lund University) for their technical assistance. The study was supported by research grants from the Swedish Heart–Lung Foundation and the Westerström Foundation.

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