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Ultrasonic Characterization of the Pulmonary Venous Wall
Echographic and Histological Correlation

José Angel Cabrera, MD, PhD; Damián Sánchez-Quintana, MD, PhD; Jerónimo Farré, MD, PhD, FESC; Felipe Navarro, MD; José Manuel Rubio, MD; Fernando Cabestrero, MD; Robert H. Anderson, MD, FRCPath; Siew Yen Ho, PhD, FRCPath

Background—Pulmonary vein isolation with radiofrequency catheter ablation techniques is used to prevent recurrences of human atrial fibrillation. Visualization of the architecture at the venoatrial junction could be crucial for these ablative techniques. Our study assesses the potential for intravascular ultrasound to provide this information.

Methods and Results—We retrieved 32 pulmonary veins from 8 patients dying from noncardiac causes. We obtained cross-sectional intravascular ultrasound (IVUS) images with a 3.2F, 30-MHz ultrasound catheter at intervals on each vein. Histological cross-sections at the intervals allowed comparisons with ultrasonic images. The pulmonary venous wall at the venoatrial junction revealed a 3-layered ultrasonic pattern. The inner echogenic layer represents both endothelium and connective tissue of the media (mean maximal thickness, 1.4±0.3 mm). The middle hypoechoic stratum corresponds to the sleeves of left atrial myocardium surrounding the external aspect of the venous media. This layer was thickest at the venoatrial junction (mean maximal thickness, 2.6±0.8 mm) and decreased toward the lung hilum. The outer echodense layer corresponds to fibro-fatty adventitial tissue (mean maximal thickness, 2.15±0.36 mm). We found a close agreement among the IVUS and histological measurements for maximal luminal diameter (mean difference, −0.12±1.3 mm) and maximal muscular thickness (mean difference, 0.17±0.13 mm) using the Bland and Altman method.

Conclusions—Our experimental study demonstrates for the first time that IVUS images of the pulmonary veins can provide information on the distal limits and thickness of the myocardial sleeves and can be a valuable tool to help accurate targeting during ablative procedures. (Circulation. 2002;106:968-973.)

Key Words: atrial flutter ▪ ablation ▪ imaging ▪ arrhythmia ▪ electrophysiology

Ultrasonic technology imaging has developed rapidly in the last 10 years. The original high-frequency (20 to 40 MHz) catheter-based transducer, known as intravascular ultrasound (IVUS), is used to provide detailed high-resolution images of the morphology of arterial walls and atherosclerotic plaques to better define therapeutic strategies. Recently, it has been reported that various forms of atrial fibrillation can be cured by electrically isolating the pulmonary veins from the left atrial myocardium with the guide of a circular catheter electrode that is placed inside the pulmonary veins. These ablation techniques within or around the pulmonary veins have increased the interest on the architecture of the pulmonary venous wall and venoatrial junction. Morphological studies on postmortem human hearts have analyzed the overall structure of the pulmonary veins, providing a better understanding of the extensions and arrangement of atrial myocardium over the venoatrial junction. Recently, intravascular ultrasonic imaging using low frequencies (5 to 12 MHz) has been proposed to guide positioning of ablation catheters and to document the gross anatomic variation of the pulmonary veins. The ability of IVUS to depict the composition of the pulmonary venous wall is unknown. The present in vitro experiment compares the images obtained with high-frequency cross-sectional IVUS with the histological architecture of the walls in postmortem specimens to assess the role of this ultrasound technique as a potential aid for accurate targeting of ablation lesions in and around the pulmonary veins.

Methods
We harvested 32 pulmonary veins from 8 structurally normal heart specimens from patients dying from noncardiac causes but whose
Intravascular Ultrasound

The entire fresh left atrium and pulmonary veins were immersed in purified water, and IVUS was performed using a standard 3.2F, 30-MHz ultrasound catheter (Cardiovascular Imaging Systems, SciMed/Boston Scientific) by placing the catheter in the lumen of the vein advanced over a 0.014-inch guidewire located in the distal end of the vein. This IVUS catheter used a movable transducer within the catheter sheath, which allowed accurate, reproducible translation of the transducer at the tip of the catheter when used in conjunction with a motorized pullback device (Cardiovascular Imaging Systems, SciMed/Boston Scientific). The entire lengths of the pulmonary veins from the most ostial portion of the vein (venoatrial junction), or orifices, to 2 cm distally were imaged using the motorized pullback at 1.0 mm/second. The gain and contrast were adjusted to provide optimal image quality. Images were recorded on sVHS videotapes for subsequent analysis, and marker lines were displayed for calibration proposes. The analysis was performed on still-frame images taken from the real-time images. Cross-sectional images were digitized and measured at 1-mm intervals along the entire length of the vein, corresponding to images 1.0 mm apart. The maximal lumen diameter (MLD) and the maximal thicknesses of the layers on the ultrasound images were measured and compared with those of the histological studies performed on the same heart specimens to determine if the ultrasound layers accurately reflect the architecture of the venous wall. These comparisons were made by 2 independent observers.

Histological Examination

Cross-sections showed an innermost layer of the venous wall formed by a thin endothelium overlying a media of connective tissue and smooth muscle cells (Figure 1). The maximal thickness of the inner layer at the venoatrial junction (including endothelium and media) is presented in Table 1. In 30 of 32 pulmonary veins, there was a layer of circularly or obliquely oriented myocardial bundles extending over the inner layer but separated from it by a thin plane of fibrofatty tissues. This intermediate myocardial layer was the continuations from the left atrial myocardium (myocardial sleeves) in a fine matrix composed of collagen, elastic fibers, and blood vessels (Figure 1). The intermediate layer was thickest at the venous orifices (Table 1) and progressively diminished in thickness toward the lung hilums. Table 2 shows the maximal extension of the myocardial sleeves on the pulmonary veins. In two of the veins, one right inferior and one left inferior, myocardial extensions were absent. In all veins, the outer layer was a thick adventitia composed of fibrous tissue (collagen and elastic fibers), nerves, and blood vessels (Figure 1).

Ultrasound Appearance of the Pulmonary Venous Wall

The typical IVUS image of a normal pulmonary vein at the venoatrial junction showed a circular 3-layered pattern: a thin inner echodense layer, a black intermediate layer, and the outer echodense layer. The innermost layer was always present and appeared as an echogenic band because of acoustic reflections produced from the connective tissue of the media and the thin endothelium at the interface with fluid (Figure 2). Analyzing all veins, the mean maximal thickness of the innermost layer was 1.4±0.3 mm (range, 0.12 to 1.7 mm) at the venoatrial junction, diminishing to a range between 0 to 0.3 mm at a distance of 2 cm from the orifice. At the venoatrial junction, a distinct black interface was visualized between the inner and the outer echodense layers in 30 of the 32 veins (94%). This intermediate hypoechoic layer represented the sleeves of atrial myocardium extending over the venous media (Figures 2 and 3). This intermediate
layer was thickest at the venoatrial junction, where it had a mean maximal width of $2.6\pm 0.8$ mm (range, 0 to 3.4 mm). The intermediate echolucent stratum progressively decreased in width until it disappeared at 2 cm away from the orifices. Ultrasonic imaging showed some very thin echo-reflective white lines obliquely oriented between the inner and outer layers. Comparisons with histological sections at the same levels revealed that these lines corresponded to connective tissue separating the fascicles of myocardial fibers (Figure 3). At the venoatrial junction, two veins showed only the echodense inner and outer layers, without the black stratum between them. These were an inferior left vein and an inferior right vein that on histological examination did not have myocardial extensions (Figures 2C and 2D). Always present, and external to the middle layer, was a third echodense band representing the adventitial fibro-fatty epicardium and peri-adventitial tissues, which exhibited a characteristic “onion skin” pattern (Figure 4). Table 1 shows the mean maximal thickness of this outermost layer at the venoatrial junction. More distally, the ultrasonic appearance of the pulmonary vein was homogeneous with absence of intermediate layer (Figure 4). The mean maximal lumen diameters determined by IVUS ($12.5\pm 3$ mm; range, 9 to 22 mm) were not significantly different from those obtained by histological sections ($12.6\pm 2.7$ mm; range, 8 to 20 mm).

Figure 5 shows the levels of agreement between IVUS and histological measurements for the maximal luminal diameter (MLD) and the maximal thickness of the 3 layers identified in cross-sections of the wall at the venoatrial junction using the Bland-Altman method. Ultrasonic images depict the presence of myocardial sleeves in all of the 30 veins in which the latter existed on the histological sections. In addition, the IVUS maximal width of this echolucent intermediate layer at the venoatrial junction agreed closely with the histological measurements for maximal myocardial thickness at the same level with a mean absolute difference of $0.17\pm 0.13$ mm (limits of agreement between $+0.42$ and $-0.09$ mm).

**Discussion**

**Major Findings**

Previous studies have shown the ability of intravascular ultrasound to depict the architecture of the arterial wall in the experimental and clinical settings. Our experimental study demonstrates for the first time that high-frequency IVUS provides information on the architecture of the pulmonary venous wall. The normal ultrasonic cross-sectional appearance of the pulmonary vein visualized experimentally with a standard 3.2F, 30-MHz ultrasound catheter consisted of a 3-layered pattern. There was an inner echogenic layer, an intermediate echolucent stratum, and an outer echodense band that accurately reflected the histological architecture of the pulmonary venous wall. IVUS enabled us to define not only the presence but also the absence of myocardial extensions at the most ostial portion of the pulmonary veins (Figure 2). These results might form a basis for future clinical applications.

**Ultrasonic Definition of the Myocardial Sleeves**

The pulmonary venous wall in the human comprises a thin endothelium and media consisting of fibrous tissue and smooth muscle, with or without an irregular middle layer of atrial myocardial tissue, and a thick fibro-fatty adventitia on the outside (Figure 1). The presence of myocardial sleeves extending from the left atrium onto the pulmonary veins toward the lung is well documented. This atrial myocardium, with bundles arranged in varying orientations, passes between the adventitia and media of the venous wall. Presently, the myocardial sleeves of the pulmonary venous wall can only be visualized by histological examinations in post-mortem specimens. Angiography and low-frequency (5- to 12-MHz) IVUS have been used to visualize the pulmonary veins and to detect the presence of stenosis after ablation procedures. Low-frequency ultrasonic transducers have large acoustic penetration but, owing to their low near-field spatial resolution, cannot provide information on the structure of the venous wall. Using a 30-MHz ultrasound catheter, we have demonstrated its ability to depict the trilaminar architecture of the venous wall, with the middle hypoechogenic stratum representing the sleeves of atrial myocardium. The acuity of detecting an echolucent interface between the inner and outer echodense layers depends on the presence and thickness of the myocardial sleeves, as seen in the histological study of the same pulmonary vein.

**TABLE 1. Mean Maximal Thickness of the 3 Layers Identified in Cross-Sections of the Wall of All the Pulmonary Veins at the Venoatrial Junction**

<table>
<thead>
<tr>
<th>Thickness, mm</th>
<th>Inner Layer</th>
<th>Intermediate Layer</th>
<th>Outer Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravascular ultrasound imaging</td>
<td>$1.4\pm 0.3$ ($0.12$ to $1.7$)</td>
<td>$2.6\pm 0.8$ ($0$ to $3.4$)</td>
<td>$2.15\pm 0.36$ ($0.5$ to $2.6$)</td>
</tr>
<tr>
<td>Histological study</td>
<td>$1.2\pm 0.3$ ($0.05$ to $1.3$)</td>
<td>$2.4\pm 0.8$ ($0$ to $3.2$)</td>
<td>$1.9\pm 0.3$ ($0.3$ to $2.3$)</td>
</tr>
</tbody>
</table>

*Values are mean±SD (range).*

**TABLE 2. Extension (Length) of the Myocardial Sleeves on 4 Pulmonary Veins**

<table>
<thead>
<tr>
<th>Length, mm</th>
<th>LSPV</th>
<th>RSPV</th>
<th>LIPV</th>
<th>RIPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravascular ultrasound imaging</td>
<td>$11.5\pm 3.5$ ($4.3$ to $14.7$)</td>
<td>$8.5\pm 3.5$ ($2.3$ to $11.7$)</td>
<td>$5.8\pm 3.3$ ($0$ to $10.5$)</td>
<td>$3.6\pm 3.4$ ($0$ to $8.5$)</td>
</tr>
<tr>
<td>Histological study</td>
<td>$10.7\pm 4.5$ ($3.3$ to $13.6$)</td>
<td>$7.8\pm 4.3$ ($2.1$ to $10.5$)</td>
<td>$5.2\pm 2.8$ ($0$ to $9.8$)</td>
<td>$3.2\pm 3.3$ ($0$ to $7.8$)</td>
</tr>
</tbody>
</table>

*Values are mean±SD (range). LIPV indicates left inferior pulmonary vein; LSPV, left superior pulmonary vein; RIPV, right inferior pulmonary vein; and RSPV, right superior pulmonary vein.*
previously observed, the myocardial sleeves were thicker at the venoatrial junction than more peripherally toward the pulmonary hilum. Ultrasonic imaging provides accurate estimates of the thickness and cross-sectional distribution of the myocardial sleeves along the pulmonary venous wall when compared with the histological sections obtained at similar levels. Because the thickness of the myocardial extension into the pulmonary veins is not uniform, we have measured and compared only the maximal thickness. At some points, the ultrasonic appearance is altered by the presence of areas of fibrosis within the myocardial sleeves. In these areas, ultrasound showed very thin echoreflective lines crossing the intermediate hypoechogenic stratum, making it difficult to measure the thickness of the sleeves.

There are some controversies regarding differences in the histological architecture of the myocardial sleeves in post-mortem hearts between normal veins and those from patients known to have suffered atrial fibrillation. Based on the results of our experimental study, high-resolution IVUS may in the future prove a valuable method to assess these differences in living patients.

**Intracardiac Echocardiography to Guide Ablation of Atrial Fibrillation**

Previous studies have suggested that intracardiac echocardiography using a 5- to 12-MHz ultrasound transducer is a useful tool during catheter ablation of cardiac arrhythmias. In contrast with fluoroscopy, ultrasound allows direct endocardial visualization, facilitating a precise spotting of the ablation catheter in relation to important anatomic landmarks and ensuring stable endocardial contact.

Radiofrequency catheter ablation is used to modify the arrhythmogenic substrate in patients with paroxysmal and persistent atrial fibrillation. Recent studies have proposed to guide the positioning of the ablation catheter and to document the gross morphological features of intracardiac structures, such as anatomic variations of the pulmonary veins. In addition, ultrasound provides clear visualization of the interatrial septum and oval fossa, serving to guide transseptal puncture without complications. MRI has also been used to study pulmonary venous anatomy and revealed a dilation of the superior pulmonary veins in patients with atrial fibrillation. The muscular architecture of the pulmonary veins, nonetheless, cannot be assessed with the above-mentioned methods. Our in vitro study shows that high-resolution IVUS imaging overcomes this limitation, providing accurate information on the cross-sectional pres-
ence or absence of myocardial sleeves at the venoatrial junction and along the pulmonary venous wall.

A previous in vivo study in dogs showed the utility of low-frequency intracardiac echocardiography to evaluate tissue contact, tissue heating, and lesion size during radiofrequency catheter ablation. High-frequency IVUS may help us to select the optimal levels of radiofrequency energy applied in different areas of the pulmonary venoatrial junction in catheter ablation procedures for patients with atrial fibrillation. Present recommendations regarding this matter are not based on experimental data but on conservative estimates from previous bad experiences resulting in pulmonary vein stenosis. Finally, high-frequency pulmonary vein ultrasound imaging, by identifying the extent and width of myocardial sleeves, might serve as a guide to target the application of radiofrequency pulses.

Limitations

Although our heart specimens were structurally normal, we were unable to confirm functional normality. Furthermore, there are no data available comparing echogenicity of pulmonary venous wall in perfused blood with that in water. Nevertheless, our in vitro studies performed in water should be comparable, because at the arterial level, the acoustic behavior of vessel walls is similar in vitro (using water or saline) and in vivo. Our IVUS measurements made on unfixed tissues may differ from in vivo IVUS dimensions of the pulmonary vein lumen because of mechanical deformation produced by the in vitro setup. Additional limitations of our study could potentially be caused by an eccentric or angulated position of the IVUS catheter within the venous lumen. When the IVUS catheter is angulated in relation to the vessel’s longitudinal axis, the diameter of the lumen will be overestimated. An eccentric position of the IVUS catheter can produce a blooming effect that may alter the measurement of the echo-dense layers of the venous wall.

In vivo studies of high-frequency IVUS will be needed to validate the application of this technique in patients with atrial fibrillation whose pulmonary veins might be larger than those of the normal population and to assess its usefulness during ablation procedures, including an evaluation of the ease of exploring the veins with the ultrasound catheter. Should the pulmonary veins have much larger diameters than our specimens, the characterization of the wall architecture may be more difficult because of the short acoustic penetration of high-frequency ultrasound. This limitation will be overcome in the future by transducers that are able to switch to different ultrasonic frequencies, thus providing simultaneous visualization of intracardiac structures and components of the atrial and venous walls.

Finally, the potential value of IVUS imaging to guide radiofrequency catheter ablation in atrial fibrillation may depend on the selected approach. Circumferential ablation around the pulmonary vein orifices, as performed by Pappone et al., will not benefit from an IVUS examination aimed at defining the myocardial content at the venoatrial junction. Should the procedure require approaching the most ostial portion of the veins, then IVUS can be helpful.

Acknowledgments

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