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Circulation 2002;106;968-973; originally published online Jul 29, 2002;
DOI: 10.1161/01.CIR.0000026397.78200.C4
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

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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 hypoechogenic 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.[1–5] Recently, it has been reported that various forms of atrial fibrillation can be cured by electrically isolating the pulmonary veins with radiofrequency catheter-ablation techniques.[6–13] Whereas one of the aforementioned techniques is aimed at creating a circumferential ablation line around the pulmonary venous orifices,[10,12] the other approach tries to disconnect the atrial myocardial sleeves inside the pulmonary veins from the left atrial myocardium with the guide of a circular catheter electrode that is placed inside the pulmonary veins.[9,11,13] 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.[14–18] 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.[19,20] 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

Received March 14, 2002; revision received May 30, 2002; accepted May 31, 2002.

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Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000026397.78200.C4**

clinical histories were unknown to us. They were from 6 men and 2 women aged 53±15 years at the time of death (range, 36 to 73 years). Of the 8 hearts, 2 had a common orifice of the left veins at the venoatrial junction. Immediately after autopsy, which was performed within 24 hours of death, the left atrium, pulmonary veins, and part of the lung hilum were isolated from the fresh heart.

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 Analysis

After completion of the IVUS imaging, the isolated pulmonary veins were fixed for at least 1 week in 10% neutral buffered formaldehyde, and then cross-sections were cut at 3-mm intervals along each isolated pulmonary vein, which had a mean length of 2 cm. For practical reasons, the 3-mm-thick segments were the thinnest possible for histological processing. Serial cross-sections 10 μm thick were cut from each segment of pulmonary vein, and alternate sections were stained with Masson's trichrome and with elastic van Gieson stain. The maximal thickness of the adventitial, myocardial, and subendothelial components of the venous wall were measured from serial cross-sectional light microscopy images at ≈1-mm intervals. Histological analysis of the pulmonary venous architecture was performed by 2 investigators blinded to the results obtained by IVUS.

Statistical Analysis

All values are expressed as mean±SD. Limits of agreement between IVUS and histological measurement at the venoatrial junction are reported as mean±2 SD of differences from paired measurement (Bland and Altman analysis).[21] Comparison of groups with continuous variables was performed with an unpaired Student's t test. $P<0.05$ was considered statistically significant.

Results

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).

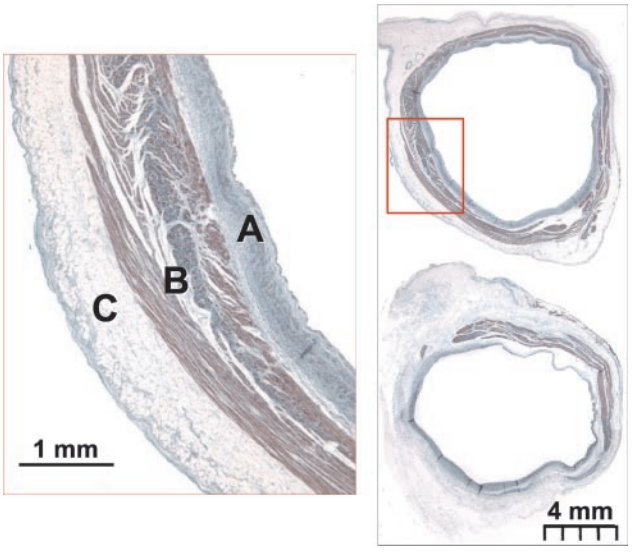

Figure 1. Histological cross-sections through left superior and inferior pulmonary veins in a normal specimen from a 68-year-old man taken 3 mm from the venoatrial junction. The left panel is a magnification of the venous wall in the left superior pulmonary vein. The venous wall comprised a thin endothelium and media of connective tissue and smooth muscles (A), an irregular intermediate layer of a sleeve of left atrial myocardium (B) with fibrillar connective tissue between the muscular bundles, and an irregular outer adventitial layer (C) composed mainly of fibrous tissue, nerves, and blood vessels. Masson's trichrome stain.

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 hypoechogenic layer represented the sleeves of atrial myocardium extending over the venous media (Figures 2 and 3). This intermediate

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

	Thickness, mm		
	Inner Layer	Intermediate Layer	Outer Layer
Intravascular ultrasound imaging	1.4±0.3 (0.12 to 1.7)	2.6±0.8 (0 to 3.4)	2.15±0.36 (0.5 to 2.6)
Histological study	1.2±0.3 (0.05 to 1.3)	2.4±0.8 (0 to 3.2)	1.9±0.3 (0.3 to 2.3)

Values are mean±SD (range).

layer was thickest at the venoatrial junction, where it had a mean maximal width of 2.6±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 echoreflective 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±3 mm; range, 9 to 22 mm) were not significantly different from those obtained by histological sections (12.6±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 at the venoatrial junction using the Bland-Altman method.[21] Ultrasound 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±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.[1,3–5] 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.[14–17] 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[22–24] and low-frequency (5- to 12-MHz) IVUS[19,20] 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. As

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

	Length, mm			
	LSPV	RSPV	LIPV	RIPV
Intravascular ultrasound imaging	11.5±3.5 (4.3 to 14.7)	8.5±3.5 (2.3 to 11.7)	5.8±3.3 (0 to 10.5)	3.6±3.4 (0 to 8.5)
Histological study	10.7±4.5 (3.3 to 13.6)	7.8±4.3 (2.1 to 10.5)	5.2±2.8 (0 to 9.8)	3.2±3.3 (0 to 7.8)

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.

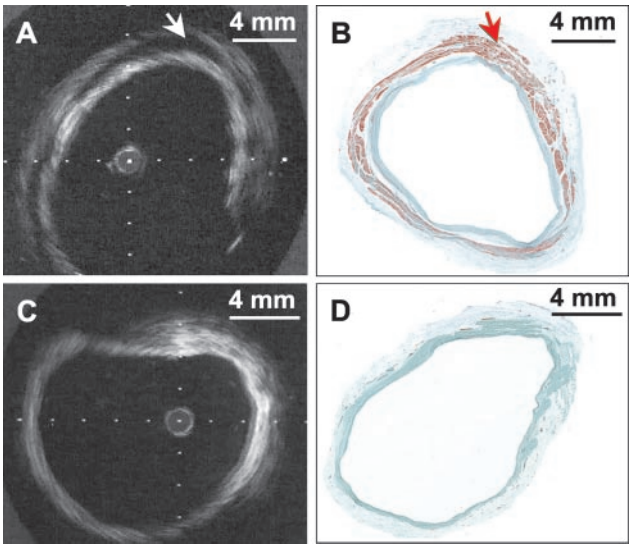

Figure 2. A and C, IVUS cross-sectional images obtained from a left inferior pulmonary vein of a 60-year-old man and a right inferior pulmonary vein of a 55-year-old woman. B and D, Corresponding histological sections (Masson's trichrome stain). Panels A and B show a representative example of the typical situation (the middle layer thicker is than the inner and outer bands). Panels C and D present an example showing the lack of intermediate layer attributable to the absence of myocardial extension into the pulmonary vein.

previously observed,[17] 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,[17] we have measured and compared only the maximal thickness. At some points, the ultrasonic appearance is altered by the presence of areas of

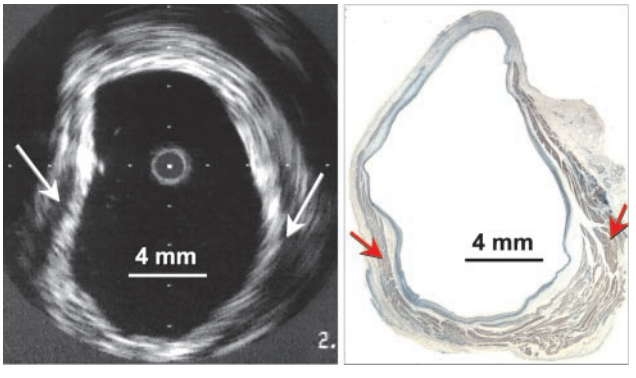

Figure 3. Echographic cross-section obtained in vitro from a right superior pulmonary vein of a specimen from a 70-year-old man (left) with corresponding histological section (right). The irregular middle layer in the histological section consists of a thick sleeve of atrial myocardium measuring 3 mm at the anteroinferior quadrant of this vein. The corresponding ultrasound image is a thick hypoechogenic stratum (arrows). In this case, the intermediate stratum is thicker than the inner and outer layers. This is the most prevalent cross-sectional ultrasonic pattern at the venoatrial junctions.

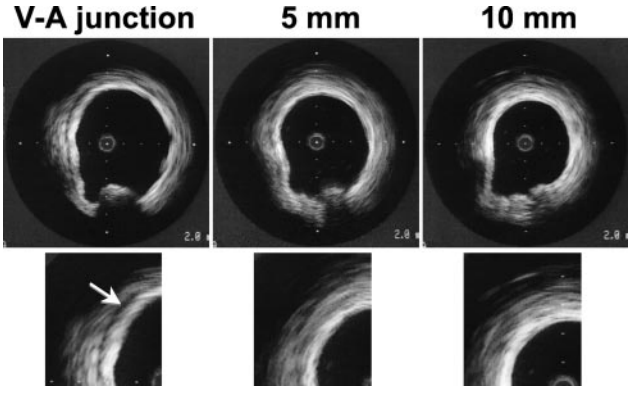

Figure 4. Intravascular ultrasound cross-sections of a left superior pulmonary vein obtained in vitro at the venoatrial (V-A) junction and 5 and 10 mm from the junction in a specimen of a 30-year-old woman. The lower panels are details of the upper sections. Note the presence of a well-defined echolucent stratum at the venoatrial junction (arrow). At 5 mm from the venoatrial junction, this middle hypoechogenic layer is less evident and becomes very irregular. More distally (10 mm), the ultrasonic appearance of the pulmonary venous wall was homogeneous without a defined intermediate layer.

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 postmortem hearts between normal veins and those from patients known to have suffered atrial fibrillation.[16,18] 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.[19,20] 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.[6–13] Recently, IVUS has been 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.[19,20,25,26] In addition, ultrasound provides clear visualization of the interatrial septum and oval fossa, serving to guide transseptal puncture without complications.[25,26] MRI has also been used to study pulmonary venous anatomy and revealed a dilation of the superior pulmonary veins in patients with atrial fibrillation.[27] 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-

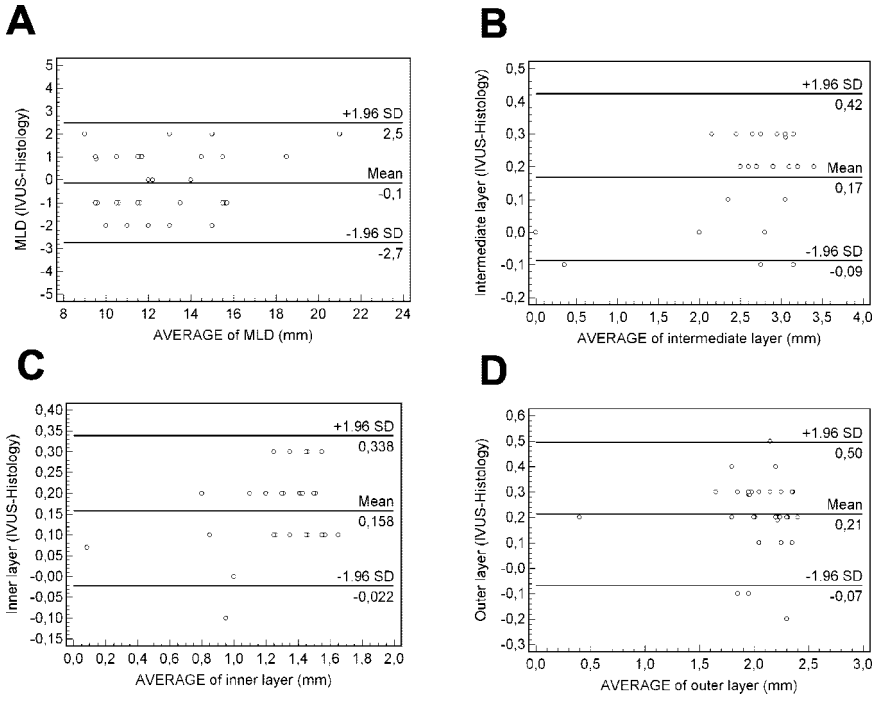

Figure 5. Comparison of maximal lumen diameter (MLD) (A) and maximal thickness of the intermediate (B), inner (C), and outer (D) layers (Bland-Altman analysis).[21] Differences are given in absolute values.

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.[28] 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.[9,11,13] 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.[1] 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.[3]

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[22,23] 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,[10,12] 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,[6–9,11,13] then IVUS can be helpful.

Acknowledgments

This study was supported by grants from FIS 01/0252 (to Drs Cabrera and Farré), Junta de Extremadura (grant IPR00A037 to Dr Sánchez-Quintana), and the Royal Brompton and Harefield Hospital Charitable Fund (to Dr Ho).

References

1. Pignoli P, Tremoli E, Poli A, et al. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation*. 1986;74:1399–1406.

2. Gussenhoven EJ, Essed CE, Lancee CT, et al. Arterial wall characteristics determined by intravascular ultrasound imaging: an in vitro study. *J Am Coll Cardiol*. 1989;14:947–952.
3. Nishimura RA, Edwards WD, Warnes CA, et al. Intravascular ultrasound imaging: in vitro validation and pathologic correlation. *J Am Coll Cardiol*. 1990;16:145–154.
4. Tobis JM, Mallery J, Mahon D, et al. Intravascular ultrasound imaging of human coronary arteries in vivo: analysis of tissue characterizations with comparison to in vitro histological specimens. *Circulation*. 1991;83: 913–926.
5. Nissen SE, Yock P. Intravascular ultrasound: novel pathophysiological insights and current clinical applications. *Circulation*. 2001;103: 604–616.
6. Haïssaguerre M, Jaïs P, Shah DC, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N Engl J Med*. 1998;339:659–666.
7. Haissaguerre M, Jais P, Shah DC, et al. Catheter ablation of chronic atrial fibrillation targeting the reinitiating triggers. *J Cardiovasc Electrophysiol*. 2000;11:2–10.
8. Chen SA, Tai CT, Tsai CF, et al. Radiofrequency catheter ablation of atrial fibrillation initiated by pulmonary vein ectopic beats. *J Cardiovasc Electrophysiol*. 2000;11:218–227.
9. Haissaguerre M, Jais P, Shah DC, et al. Electrophysiological end point for catheter ablation of atrial fibrillation initiated from multiple pulmonary venous foci. *Circulation*. 2000;101:1409–1417.
10. Pappone C, Rosanio S, Oreto G, et al. Circumferential radiofrequency ablation of pulmonary vein ostia. *Circulation*. 2000;102:2619–2628.
11. Haïssaguerre M, Shah DC, Jaïs P, et al. Electrophysiological breakthroughs from the left atrium to the pulmonary veins. *Circulation*. 2000; 102:2463–2465.
12. Pappone C, Oreto G, Rosanio S, et al. Atrial electroanatomic remodeling after circumferential radiofrequency pulmonary vein ablation: efficacy of an anatomic approach in a large cohort of patients with atrial fibrillation. *Circulation*. 2001;104:2539–2544.
13. Oral H, Knight BP, Tada H, et al. Pulmonary vein isolation for paroxysmal and persistent atrial fibrillation. *Circulation*. 2002;105: 1077–1081.
14. Nathan H, Eliakim M. The junction between the left atrium and the pulmonary veins: an anatomic study of human hearts. *Circulation*. 1966; 34:412–422.
15. Ho SY, Sánchez-Quintana D, Cabrera JA, et al. Anatomy of the left atrium: implication for radiofrequency ablation of atrial fibrillation. *J Cardiovasc Electrophysiol*. 1999;10:1525–1533.
16. Saito T, Waki K, Becker AE. Left atrial myocardial extension onto pulmonary veins in human: anatomic observations relevant for atrial arrhythmias. *J Cardiovasc Electrophysiol*. 2000;11:888–894.
17. Ho SY, Cabrera JA, Tran VH, et al. Architecture of the pulmonary veins: relevance to radiofrequency ablation. *Heart*. 2001;86:265–270.
18. Tagawa M, Higuchi K, Chinushi M, et al. Myocardium extending from the left atrium onto the pulmonary veins: a comparison between subjects with and without atrial fibrillation. *Pacing Clin Electrophysiol*. 2001;24: 1459–1463.
19. Chu E, Fitzpatrick AP, Chin MC, et al. Radiofrequency catheter ablation guided by intracardiac echocardiography. *Circulation*. 1994;89: 1301–1305.
20. Cooper JM, Epstein LM. Use of intracardiac echocardiography to guide ablation of atrial fibrillation. *Circulation*. 2001;104:3010–3013.
21. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1:307–310.
22. Lin WS, Prakash VS, Tai CT, et al. Pulmonary vein morphology in patients with paroxysmal atrial fibrillation initiated by ectopic beats originating from the pulmonary veins: implication for catheter ablation. *Circulation*. 2000;101:1274–1281.
23. Yamane T, Shah DC, Hocini M, et al. Pulmonary vein aneurysm in association with arrhythmogenic foci in a patient with focally initiated atrial fibrillation. *J Cardiovasc Electrophysiol*. 2000;11:715.
24. Scanavacca MI, Kajita LJ, Vieira M, et al. Pulmonary vein stenosis after catheter ablation of focal atrial fibrillation. *J Cardiovasc Electrophysiol*. 2000;11:677–681.
25. Morton JB, Sander P, Byrne MJ, et al. Phased-array intracardiac echocardiography to guide radiofrequency ablation in the left atrium and the pulmonary vein ostium. *J Cardiovasc Electrophysiol*. 2001;12:343–348.
26. Daoud EG, Kalbfleisch SJ, Hummel JD. Intracardiac echocardiography to guide transseptal left heart catheterisation for radiofrequency catheter ablation. *J Cardiovasc Electrophysiol*. 1999;10:358–363.
27. Tsao HM, Yu WC, Cheng HC, et al. Pulmonary vein dilation in patients with atrial fibrillation: detection by magnetic resonance imaging. *J Cardiovasc Electrophysiol*. 2001;12:809–813.
28. Kalman JM, Fitzpatrick AP, Olgin JE, et al. Biophysical characteristics of radiofrequency lesion formation in vivo: dynamics of catheter tip-tissue contact evaluated by intracardiac echocardiography. *Am Heart J*. 1997; 133:8–18.