Journal of the American College of Cardiology
© 2012 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 59, No. 10, 2012
ISSN 0735-1097/$36.00
doi:10.1016/j.jacc.2011.09.076

Histopathologic Characterization of Chronic Radiofrequency Ablation Lesions for Pulmonary Vein Isolation

Marcin Kowalski, MD,* Margaret M. Grimes, MD,† Francisco J. Perez, MD,* David N. Kenigsberg, MD,* Jayanthi Koneru, MD,* Vigneshwar Kasirajan, MD,‡ Mark A. Wood, MD,* Kenneth A. Ellenbogen, MD*

Richmond, Virginia

Objectives This study describes the histopathologic and electrophysiological findings in patients with recurrence of atrial fibrillation (AF) after pulmonary vein (PV) isolation who underwent a subsequent surgical maze procedure.

Background The recovery of PV conduction is commonly responsible for recurrence of AF after catheter-based PV isolation.

Methods Twelve patients with recurrent AF after acutely successful catheter-based antral PV isolation underwent a surgical maze procedure. Full-thickness surgical biopsy specimens were obtained from the PV antrum in areas of visible endocardial scar. Before biopsy, intraoperative epicardial electrophysiological recordings were taken from each PV using a circular mapping catheter.

Results Twenty-two PVs were biopsied from the 12 patients 8 ± 11 months after ablation. Eleven of the 22 specimens (50%) revealed transmural scar, and 11 (50%) showed viable myocardium with or without scar. Each biopsy specimen demonstrated evidence of injury, most commonly endocardial thickening (n = 21 [95%]) and fibrous scar (n = 18 [82%]). Seven of the 22 specimens (32%) showed conduction block at surgery. Transmural scar was more likely to be seen in the biopsy specimens from the PVs with conduction block than in specimens from the PVs showing reconnection. However, viable myocardium alone or mixed with scar was seen in 2 specimens from PVs with conduction block.

Conclusions PVs showing electrical reconnection after catheter-based antral ablation frequently reveal anatomic gaps or nontransmural lesions at the sites of catheter ablation. Nontransmural lesions are noted in some PVs with persistent conduction block, suggesting that lesion geometry may influence PV conduction. The histological findings show that nontransmural ablation can produce a dynamic cellular substrate with features of reversible injury. Delayed recovery from injury may explain late recurrences of AF after PV isolation. (J Am Coll Cardiol 2012;59: 930–8) © 2012 by the American College of Cardiology Foundation

Catheter-based electrical isolation of the pulmonary veins (PVs) has emerged as an important therapy for patients with drug-refractory atrial fibrillation (AF) (1). The success rate at 12 months for patients at experienced centers is approximately 70%, with wide variability in reported successful outcomes (2). Recovery of PV conduction is considered to be the most common reason for recurrent AF after an initially successful procedure (3–5). It has been speculated that the recovery of PV conduction is due to failure to create transmural lesions or to anatomic gaps in the ablation line(s). This conjecture has not been confirmed by using histological studies of PVs after unsuccessful PV isolation in humans. In the present study, we describe the histopathologic and electrophysiological findings of PVs in patients undergoing a surgical maze procedure for recurrent AF after initially successful catheter-based PV isolation.

See page 939

Methods

Prior ablation. The study included 12 consecutive patients with paroxysmal (n = 6) or persistent (n = 6) AF who underwent surgical maze procedures between August 2006

From the *Department of Clinical Cardiac Electrophysiology, Virginia Commonwealth University Medical Center, Richmond, Virginia; †Department of Pathology, Virginia Commonwealth University Medical Center, Richmond, Virginia; and the ‡Department of Cardiothoracic Surgery, Virginia Commonwealth University Medical Center, Richmond, Virginia. Dr. Kasirajan is a consultant for and has received research support from Atricure Inc. Dr. Wood has received clinical research support from BioSense Webster. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received April 27, 2011; revised manuscript received August 29, 2011, accepted September 27, 2011.

and January 2009 for recurrent AF after successful catheter-based PV isolation procedures. The study was approved by our institutional review board, and all patients provided written informed consent to the investigational protocol. Nine patients had undergone antral PV isolation at our institution guided by intracardiac echocardiogram (AcuNav, Siemens Medical Solutions, Mountain View, California) and circular multipolar electrode catheter recordings (Lasso, Biosense Webster, Diamond Bar, California), as described previously (4,6). These patients underwent radiofrequency (RF) ablation with a 3.5-mm tip open-irrigation catheter (ThermoCool, Biosense Webster) with power limited to 25 W on the posterior left atrium and 35 W on the remaining sites. Energy application was 20 s on the posterior left atrium and up to 45 s in anterior locations. In addition, 1 patient underwent cryoablation with an 8-mm tip catheter (CryoCath Freezor MAX, Medtronic, Montreal, Quebec, Canada) to a single PV that could not be isolated proximal to the PV ostium. In these 9 patients, isolation of all 4 PVs was demonstrated by entrance and exit block. Persistent electrical isolation of each vein was confirmed during isoprotenolol infusion up to 10 μg/min 20 min after isolation of the last PV. The other 3 patients had their procedures performed at outside institutions, and specific details about their procedure were unavailable, but it was reported that they underwent PV isolation procedures with entrance block as the electrophysiological endpoint.

Surgical procedures. A full surgical Cox maze III procedure was performed in each of the 12 patients via median sternotomy, as described previously (7,8). Before left atriotomy, a circular mapping catheter (Lasso) was placed epicardially around each PV as close to the antrum as possible. Bipolar recordings were made from the 20-pole catheter on a physiological recorder (Prucka Cardiolab, GE Healthcare, Waukesha, Wisconsin). After recordings were obtained from each PV, a left atriotomy was performed on cardiopulmonary bypass. The endocardial surface near each PV was visualized and examined for presence of scarring from the prior ablation. A full-thickness incisional biopsy specimen was obtained from locations at which the prescribed atriotomy lines crossed the prior ablation lines. Concomitant tricuspid valve annuloplasty was performed in 1 patient, mitral valve annuloplasty in 1 patient, coronary artery bypass grafting in 3 patients, patent foramen ovale closure in 2 patients, and aortic valve replacement with ascending aortic aneurysm repair due to bicuspid aortic valve in 1 patient. In 6 patients, the maze procedure was performed as the only intervention.

Abbreviations and Acronyms

AF = atrial fibrillation
PV = pulmonary vein
RF = radiofrequency

Histopathologic tissue examination. Tissue specimens were fixed in 10% neutral buffered formalin and processed to paraffin blocks. Histological sections prepared from the blocks were stained with hematoxylin and eosin and trichrome stains and examined by using light microscopy.

Statistical analysis. Baseline descriptive variables are presented as frequencies and percentages for categorical variables and mean ± SD for continuous variables. All analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, Illinois).

Table 1 Demographic Characteristics

Age (yrs)	59 ± 8
Male	11 (92)
Diabetes mellitus	1 (8)
Hypertension	7 (58)
CAD	3 (25)
Ejection fraction	50 ± 12%
Beta-blockers	10 (83)
Warfarin	11 (92)
Aspirin	6 (50)
Anti-arrhythmic drugs failed	
Amiodarone	2 (17)
Dofetilide	5 (42)
Propafenone	4 (34)
Type of AF	
Persistent	6 (50)
Paroxysmal	6 (50)
Catheters used	
ThermoCool irrigated tip catheter, 3.5 mm*	11 (17)
Chilli internally cooled tip catheter†	1 (83)

Values are mean ± SD or n (%). *One patient underwent a combined procedure using an open-irrigation 4-mm tip deflectable catheter (ThermoCool, Biosense Webster, Diamond Bar, California) and an 8-mm cryoablation catheter (CryoCath, Montreal, Quebec, Canada). †Boston Scientific, Natick, Massachusetts.
AF = atrial fibrillation; CAD = coronary artery disease.

Results

Patient characteristics. The characteristics of the 12 study patients are shown in Table 1. The majority of patients were male (92%) with a mean age of 59 ± 8 years, normal left ventricular function, and with both persistent (n = 6) and paroxysmal (n = 6) AF. Documented recurrences of AF occurred 6 ± 14 months (range 3 to 30 months) after the last RF ablation. The surgical maze procedures were performed 8 ± 11 months (range 4 to 37 months) after the catheter ablation.

Biopsy specimens. Twenty-two PVs were biopsied at the sites of dense antral scar, with a range of 1 to 4 PV biopsy specimens per patient (Table 2). The specimens were obtained from 9 right superior PVs, 3 right inferior PVs, 7 left superior PVs, and 3 left inferior PVs. The mean thickness of the biopsy specimens was 3.5 ± 1.2 mm (range 1 to 6 mm). In 2 samples, tangential sectioning precluded assessment of biopsy thickness.

Postsurgical follow-up. Of the 12 patients, 2 were followed up in other hospitals after their surgical procedures. The median follow-up duration of the remaining 10 patients was 30 months (range 6 to 60 months). Of the 10 patients who were followed up at our institution, only 1 patient had a clinical recurrence of AF necessitating therapy.

Table 2 Histology Findings

PV #	PV Location	Time to Recurrence Post-RF (Months)	Time to Biopsy (Months)	Histology Type	Electrical Connection of the PV	Fibrous Scar Present	Transmurality of the Scar	Interstitial Fibrosis	Viable Myocardium Present Anywhere in the Biopsy Specimen	Hypereosinophilia	Contraction Bands	Myocytolysis	Nuclear Pyknosis	Myocyte Hypertrophy	Endocardial Thickening
1	RS	11.80	129.60	Scar	Conducts	+	+	+	+	+	−	+	+	+	+
2	RS	6.40	13.37	Scar	Blocked	+	+	−	−	−	−	−	−	−	+
3	RI	6.40	13.37	Viable	Blocked	−	−	+	+	−	−	−	−	+	+
4	LI	2.87	3.97	Scar	Conducts	+	+	−	−	−	−	−	−	−	+
5	RS	2.87	3.97	Scar	Conducts	+	+	+	+	+	+	−	+	+	+
6	LI	2.10	12.30	Scar	Conducts	+	+	−	+	−	−	−	−	+	+
7	LS	2.10	12.30	Mixed	Conducts	+	− (50%)†	+	+	+	−	−	+	+	+
8	RI	2.10	12.30	Scar	Conducts	+	+	+	+	+	−	−	+	+	+
9	RS	2.10	12.30	Scar	Conducts	+	*	−	+	+	−	+	+	+	+
10	LS	14.23	14.23	Viable	Conducts	−	−	−	+	+	+	+	+	+	+
11	RS	0.87	14.23	Mixed	Blocked	+	− (10%)†	+	+	+	−	+	+	+	+
12	RS	2.70	4.80	Scar	Blocked	+	+	−	−	−	−	−	−	−	+
13	RI	2.70	4.80	Scar	Blocked	+	+	−	−	−	−	−	−	−	+
14	LS	0.80	1.77	Scar	Blocked	+	+	+	+	+	−	+	+	+	+
15	RS	30.00	37.23	Scar	Blocked	+	+	+	+	+	−	+	−	+	+
16	LI	5.80	6.70	Mixed	Conducts	+	− (50%)†	−	+	−	−	+	+	+	+
17	RS	5.80	6.70	Mixed	Conducts	+	− (20%)†	−	+	+	+	−	+	+	+
18	LS	5.80	6.70	Mixed	Conducts	+	− (20%)†	−	+	−	−	+	−	+	+
19	LS	7.00	20.30	Mixed	Conducts	+	− (20%)†	+	+	+	+	+	+	+	+
20	RS	7.00	20.30	Mixed	Conducts	+	*	−	+	+	+	+	+	+	+
21	LS	4.37	4.90	Viable	Conducts	−	−	−	+	−	−	−	−	+	−
22	LS	0.97	15.47	Viable	Conducts	−	−	+	+	−	−	+	+	+	+

*Tangential sectioning precludes assessment. †Thickness of the nontransmural scar compared with the wall thickness of the left atrium.
− = absent; + = present; LI = left inferior; LS = left superior; PV = pulmonary vein; RF = radiofrequency; RI = right inferior; RS = right superior.

Histological findings. Histological analysis of the biopsy samples showed findings consistent with myocardial thermal injury in every sample (Table 2). The most common evidence of injury was endocardial thickening in 21 specimens (95%), fibrous scar in 18 specimens (82%), and nuclear pyknosis in 16 specimens (73%) (Table 2). Four (18%) biopsy specimens showed only viable myocardium but no fibrous scar (Fig. 1). Seven (32%) biopsy results demonstrated nontransmural fibrous scar with adjacent viable myocardium (Fig. 2), and 11 biopsy results (50%) showed transmural fibrous scar with no viable myocardium (Fig. 3). Myocytolysis was noted in 11 (50%) specimens (Fig. 4).

PV conduction. Four patients (33%) were in sinus rhythm before the surgery, and 8 (67%) patients were in AF. Six patients demonstrated persistent conduction block in at least 1 PV. Two of these 6 patients demonstrated conduction block in all 4 veins. In 2 patients, electrical isolation was documented in 2 of the 4 veins, and 2 patients had isolation in 1 of 4 PVs. The remaining 6 patients demonstrated conduction in all 4 PVs. Of the 7 PVs with conduction block (from 5 patients), transmural

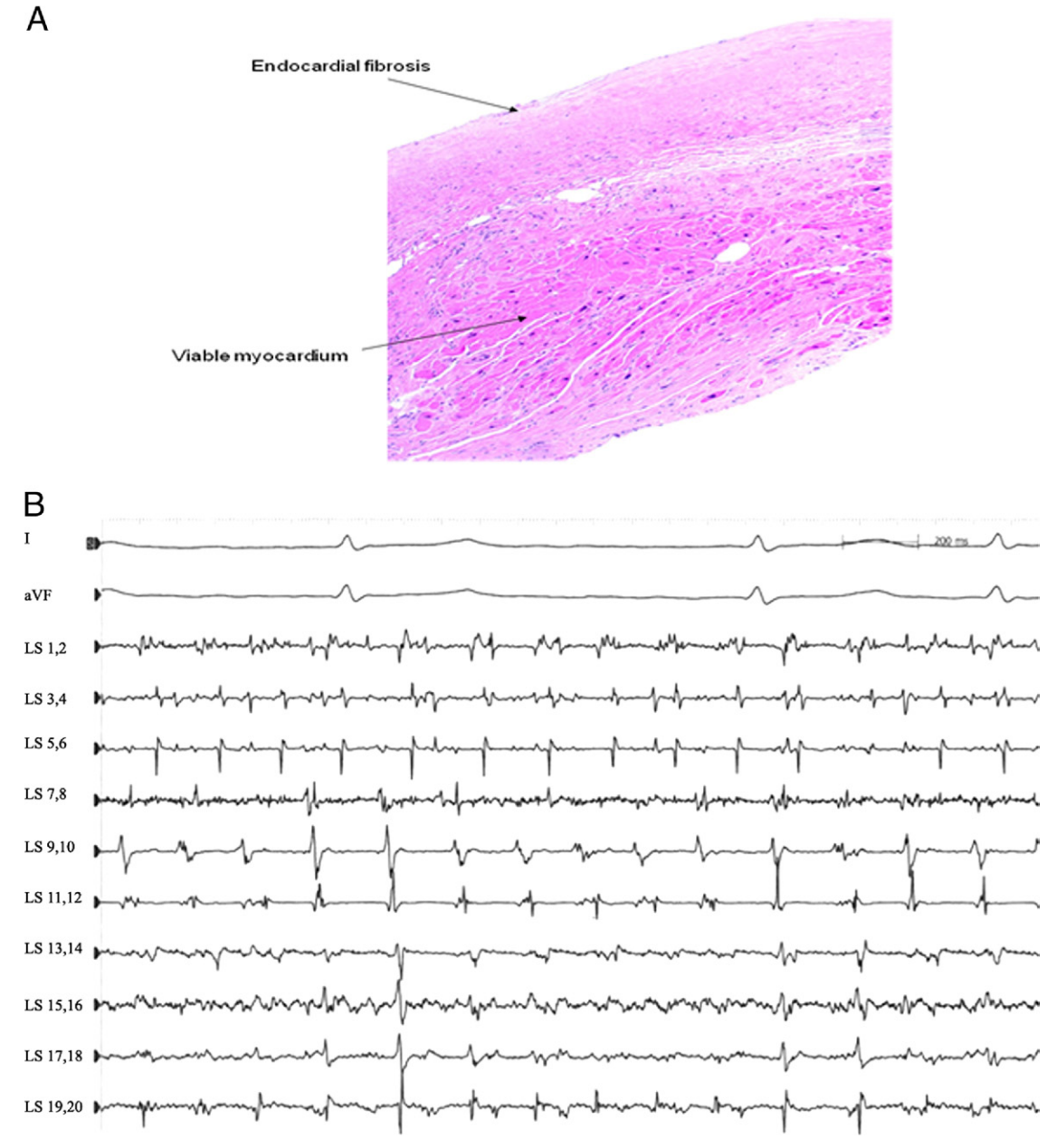

Figure 1 Histology and PV Recordings From PVs Showing Electrical Conduction

Histology showing viable myocardium, interstitial fibrosis, and no discrete fibrous scar using **(A)** hematoxylin and eosin stain and **(B)** pulmonary vein (PV) recording from a patient showing electrical conduction into PV #22 from Table 2. LS = left superior.

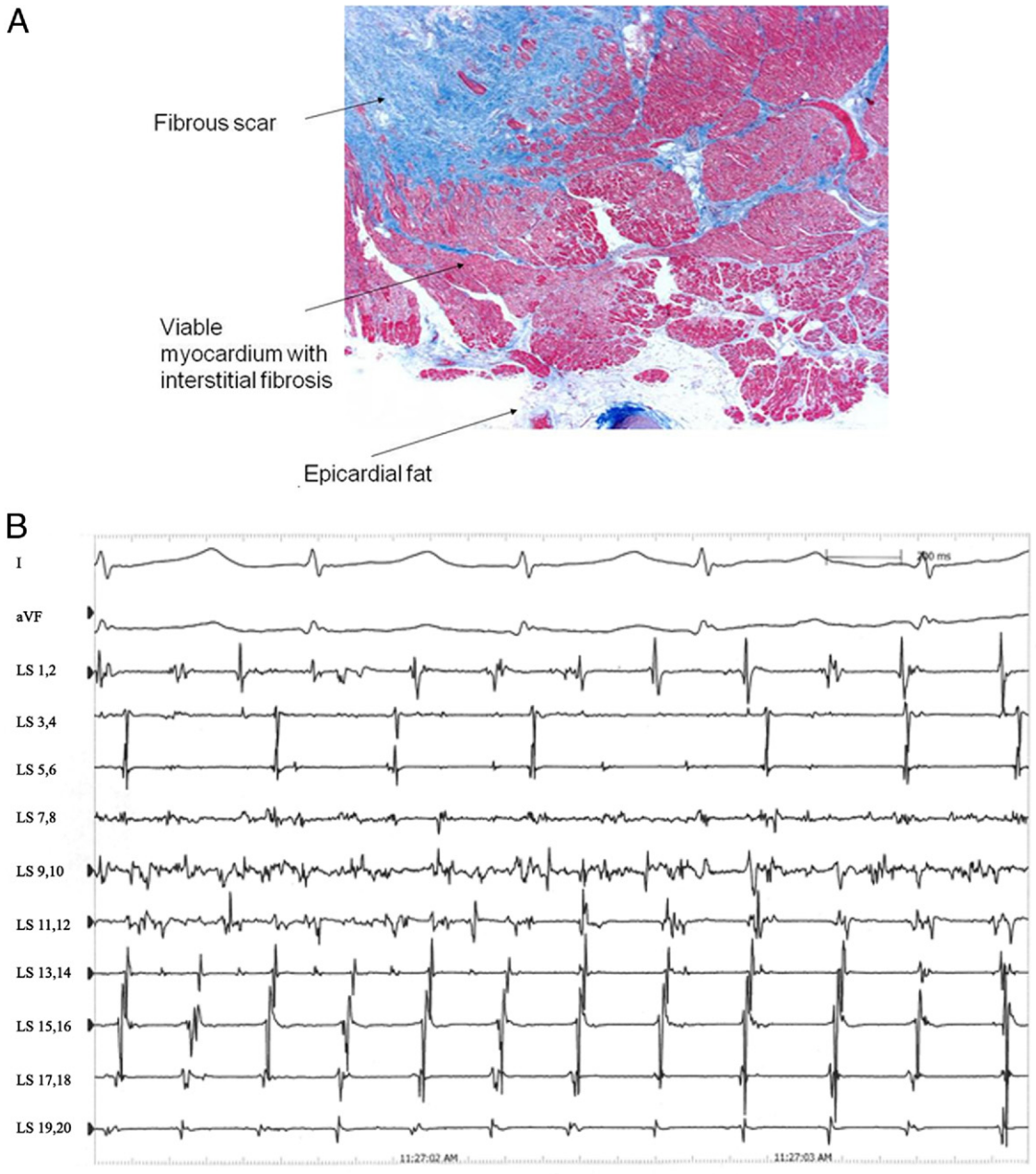

Figure 2 **Histology and PV Recordings From a Sample With Viable Myocardium and Scar Showing Electrical Reconnection**

Histology results showing combination of viable myocardium and nontransmural fibrous scar using **(A)** trichrome stain and **(B)** PV recording from the same patient showing atrial fibrillation with electrically reconnected PV #7 from Table 2. Abbreviations as in Figure 1.

scar without viable myocardium in biopsy specimens was seen in 5 PVs (71%) from 4 patients, intermixed scar and viable myocardium in 1 PV (14%) from 1 patient, and viable myocardium only in the biopsy specimen from 1 PV from 1 patient (14%).

Of the 15 PVs that recovered electrical conduction, transmural scar without viable myocardium was demonstrated in 6 PVs (40%) from 3 patients, intermixed scar and viable myocardium was seen in 6 PVs (40%) from 3 patients, and viable myocardium only was noted in 3 PVs (20%) from 3 patients. The proportion of biopsy specimens with transmural scar without viable myocardium was considerably greater for the PV with conduction block (71%) than for those PV with intact conduction (40%) (Fig. 5).

Discussion

The major findings of this study are: 1) biopsy specimens from PVs after successful catheter-based isolation frequently

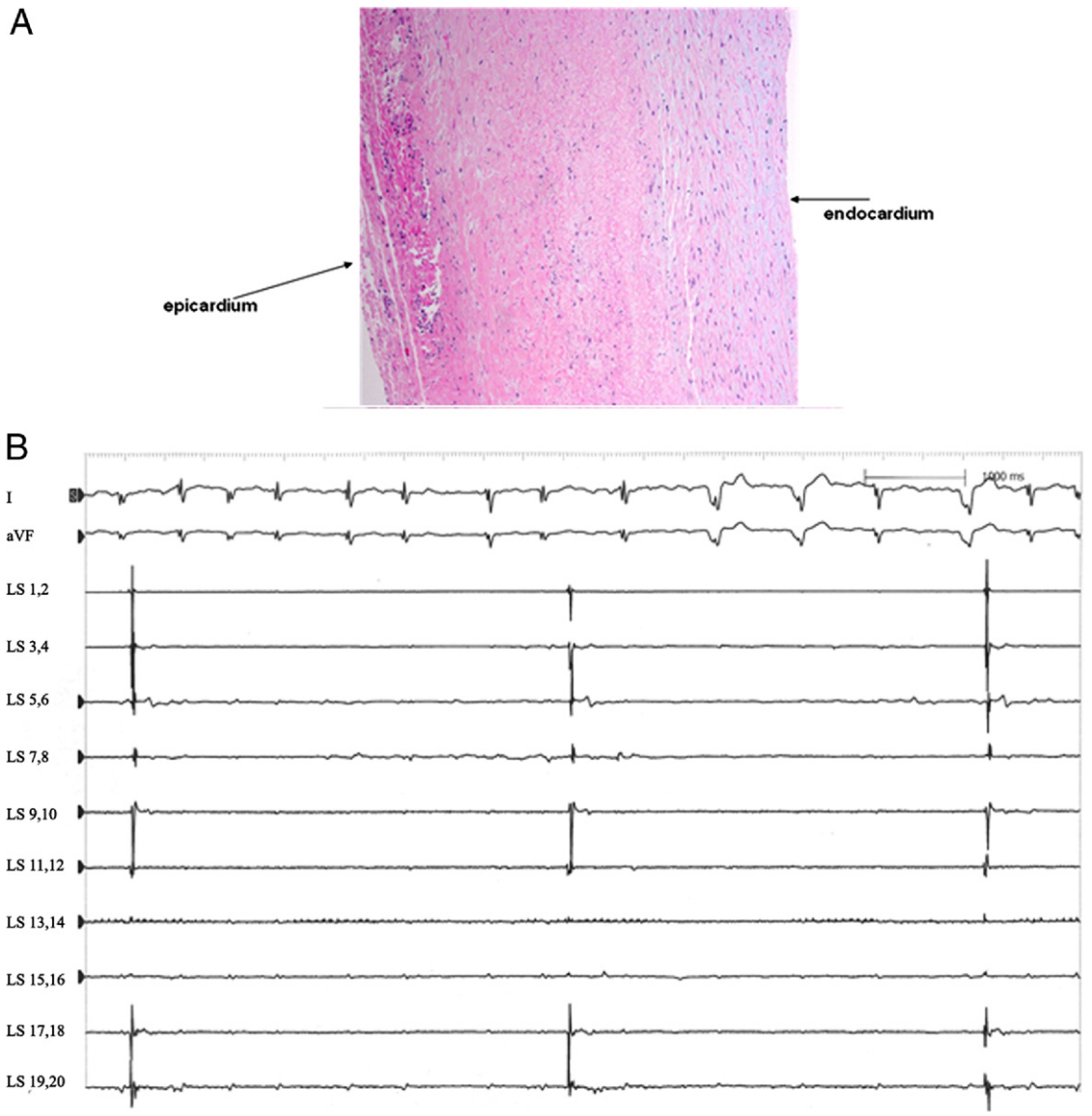

Figure 3 **Histology and PV Recordings From PVs Showing Electrical Isolation**

Histology showing transmural scar using **(A)** hematoxylin and eosin stain and **(B)** PV recording from the same patient showing electrically isolated PV potentials while patient is in atrial fibrillation from PV #2 from Table 2. Abbreviations as in Figure 1.

show nontransmural scar or gaps at the site of ablation; 2) PV conduction block may occur despite nontransmural lesions along the ablation line; and 3) the histology of antral ablation lesions is complex, with evidence of reversible cellular injury (myocytolysis) that may contribute to late recurrences of PV conduction. The rate of PV reconnection is between 50% and 81% of previously isolated PVs (3,4,9,10). Return of PV conduction after catheter-based isolation procedures is assumed to be due to failure to create permanent contiguous transmural lesions in at least part of the ablation line. The anatomic correlates to PV reconnection have not been previously demonstrated. Our findings show that lesion gaps or nontransmural lesions along the ablation line are common in patients with recurrent AF after acutely successful catheter-based PV isolation.

Very few studies have examined the histology of human atrial myocardium after AF ablation procedures. Deneke et al. (11) sampled 59 lesions at the time of intraoperative cooled tip RF catheter ablation and found lack of transmurality in 25% of all lesions. Accord et al. (12) reported the histological findings in 3 patients who died 2 to 22 days after intraoperative epicardial microwave ablation for PV isolation. At autopsy, only 3 of 13 specimens from these patients showed transmural lesions. To the best of our knowledge, there are no data on the histopathology of

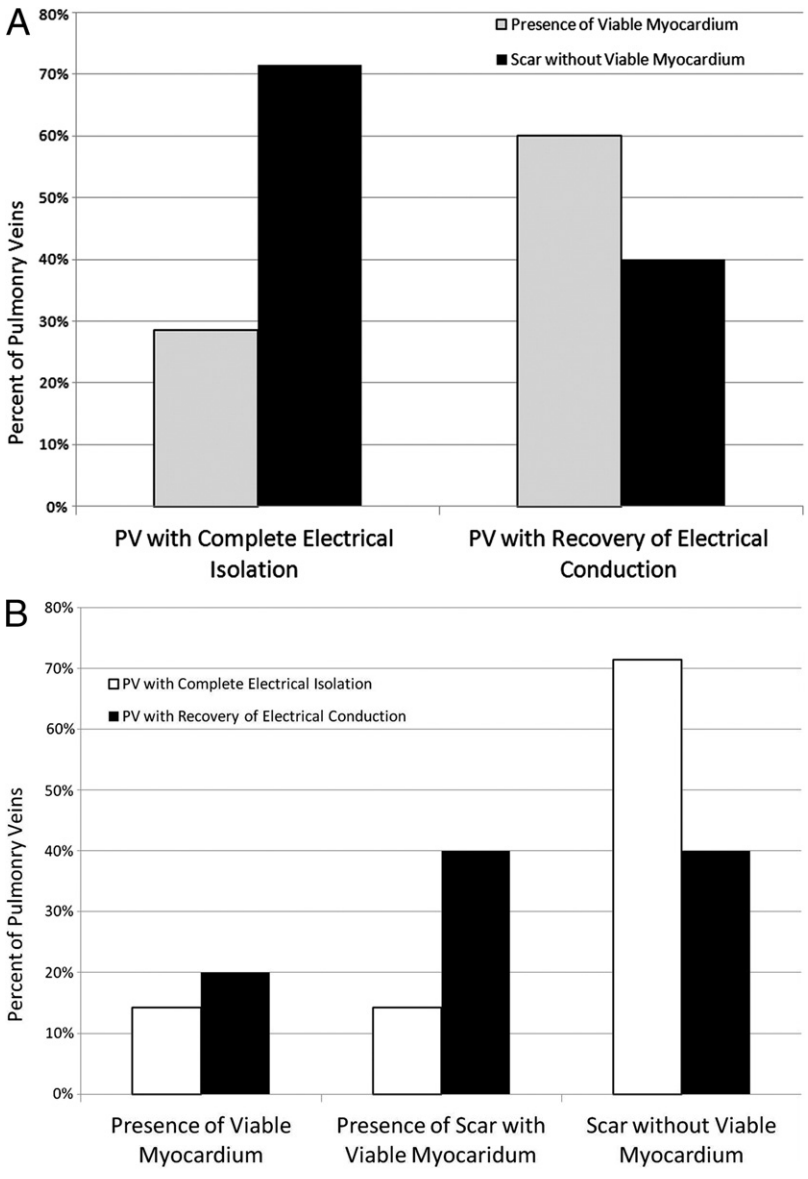

Figure 4 Evidence of Reversible Cellular Injury

Areas of myocytolysis indicated by the **arrows**. PV = pulmonary vein.

percutaneous RF ablation lesions for AF in humans. The reasons for the failure of RF catheter ablation to produce uniformly transmural lesions is not known. Clearly, there is failure to achieve uniformly lethal tissue heating during ablation. This failure may be due to poor electrode–tissue contact, insufficient power delivery, or excessive convective cooling. Tissue heating to sublethal temperatures can result in reversible loss of myocardial electrical activity (13). In addition, it is known that the maximal action potential amplitude (dV/dt) and action potential duration are all reduced within several millimeters of the edge of acute RF lesions (14). These changes are expected to involve the tissue comprising gaps in linear ablation lesions and may be responsible for rendering these discontinuities temporarily nonconductive. These changes are known to resolve after ablation, conceivably allowing a return of conduction through the gaps. Misplaced ablation lines and noncontiguous lesions could also result in failure of PV isolation. Similarly, inaccurate mapping of PV electrical activity at ablation could lead to the misdiagnosis of PV block when, in fact, conduction persists. We feel that this is unlikely because our practice is to extensively map the PV with the circular catheter starting deep within the vein and pulling back to the PV antrum. In our practice, electrogram abatement at the PV antrum is not accepted as evidence of conduction block.

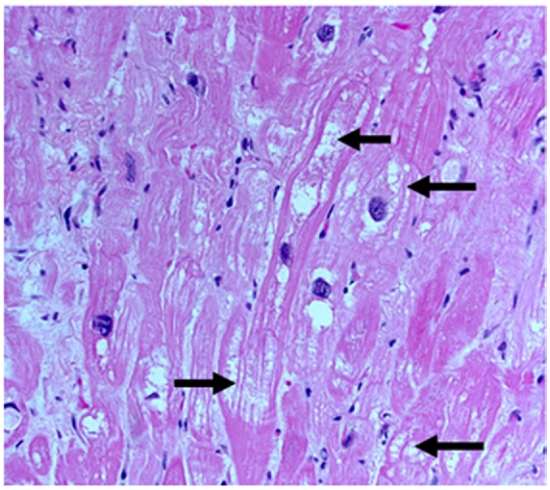

Figure 5 Comparison Between Electrical Activity and Histological Findings

(A) Graph comparing the percentage of pulmonary veins (PVs) with or without complete electrical conduction versus the presence of any viable myocardium. The PVs with complete electrical isolation had a higher percentage of veins with transmural scar without viable myocardium, whereas PVs with recovered electrical conduction had higher percentage of biopsy specimens with viable myocardium. **(B)** Graph comparing the percentage of PVs with or without complete electrical isolation versus the presence of only viable myocardium, scar with viable myocardium, or presence of only scar on biopsy results. A greater percentage of biopsy specimens demonstrating the presence of any viable myocardium (viable myocardium or viable myocardium with scar) were recovered from PVs that recovered electrical conduction, whereas the presence of transmural scar was more often found in samples obtaining from PVs with complete electrical isolation.

The finding of conduction block in PV despite nontransmural scar along the ablation line has 2 likely explanations. It is possible that the fascicles of viable tissue evident at the site of biopsy are nonconductive due to ablation at a more ostial or a more atrial site that is outside the extent of the biopsy specimen. Alternatively, the tissue geometry of the viable tissue along the ablation line is such that conduction fails due to angulation, branching, or narrowing of the propagating wave front (15).

The reason for recurrence of PV conduction that becomes manifest even years after successful PV isolation are unknown; however, the histopathologic findings in our study allow for speculation (16,17). All biopsy specimens in our study demonstrated findings consistent with thermal injury even 37 months after catheter ablation. Findings such as nuclear pyknosis are associated with apoptosis and anticipated cell death. However, the findings of myocytolysis represent potentially reversible cellular responses to injury. The tissue substrate within the ablation areas seems to be dynamic long after catheter ablation. This dynamic substrate may have the potential for late return of conduction.

Study limitations. This study has several limitations. Not all PVs could be sampled. The exact details of the procedure were unknown in 3 patients who underwent catheter ablation at outside institutions; however, all PVs were reportedly isolated after the ablation. Only a limited area of the visible scar around the PVs was sampled. Therefore, discordant findings of persistent PV conduction despite biopsy samples showing transmural scar could hypothetically be secondary to noncontiguous areas of ablative lesions being missed during sampling. The sample size is small, yet the qualitative findings of nontransmural lesions within the ablation line support previously held clinical suspicions.

Conclusions

Electrical PV reconnection was frequently seen in these study patients with recurrent AF after initially successful PV isolation. The return of PV conduction was associated with histopathologic evidence of nontransmural lesions along the ablation line.

Reprint requests and correspondence: Dr. Kenneth A. Ellenbogen, Virginia Commonwealth University Medical Center, P.O. Box 980053, Richmond, Virginia 23298-0053. E-mail: kellenbogen@mcvh-vcu.edu. or kellenbogen@pol.net.

REFERENCES

1. Fuster V, Ryden LE, Cannom DS, et al. ACC/AHA/ESC 2006 guidelines for the management of patients with atrial fibrillation—executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 Guidelines for the Management of Patients With Atrial Fibrillation). J Am Coll Cardiol 2006;48:854–906.
2. Cappato R, Calkins H, Chen SA, et al. Updated worldwide survey on the methods, efficacy, and safety of catheter ablation for human atrial fibrillation. Circ Arrhythm Electrophysiol 2010;3:32–8.
3. Haissaguerre M, Shah DC, Jais P, et al. Electrophysiological breakthroughs from the left atrium to the pulmonary veins. Circulation 2000;102:2463–5.
4. Oral H, Knight BP, Tada H, et al. Pulmonary vein isolation for paroxysmal and persistent atrial fibrillation. Circulation 2002;105:1077–81.
5. Verma A, Kilicaslan F, Pisano E, et al. Response of atrial fibrillation to pulmonary vein antrum isolation is directly related to resumption and delay of pulmonary vein conduction. Circulation 2005;112:627–35.
6. Jais P, Cauchemez B, Macle L, et al. Catheter ablation versus antiarrhythmic drugs for atrial fibrillation: the A4 study. Circulation 2008;118:2498–505.
7. Schaff HV, Dearani JA, Daly RC, Orszulak TA, Danielson GK. Cox-Maze procedure for atrial fibrillation: Mayo Clinic experience. Semin Thorac Cardiovasc Surg 2000;12:30–7.
8. Prasad SM, Maniar HS, Camillo CJ, et al. The Cox maze III procedure for atrial fibrillation: long-term efficacy in patients undergoing lone versus concomitant procedures. J Thorac Cardiovasc Surg 2003;126:1822–8.
9. Ouyang F, Ernst S, Chun J, et al. Electrophysiological findings during ablation of persistent atrial fibrillation with electroanatomic mapping and double Lasso catheter technique. Circulation 2005;112:3038–48.
10. Sauer WH, McKernan ML, Lin D, Gerstenfeld EP, Callans DJ, Marchlinski FE. Clinical predictors and outcomes associated with acute return of pulmonary vein conduction during pulmonary vein isolation for treatment of atrial fibrillation. Heart Rhythm 2006;3:1024–8.
11. Deneke T, Khargi K, Muller KM, et al. Histopathology of intraoperatively induced linear radiofrequency ablation lesions in patients with chronic atrial fibrillation. Eur Heart J 2005;26:1797–803.
12. Accord RE, van Suylen RJ, van Brakel TJ, Maessen JG. Post-mortem histologic evaluation of microwave lesions after epicardial pulmonary vein isolation for atrial fibrillation. Ann Thorac Surg 2005;80:881–7.

13. Nath S, Lynch C III, Whayne JG, Haines DE. Cellular electrophysiological effects of hyperthermia on isolated guinea pig papillary muscle. Implications for catheter ablation. Circulation 1993;88:1826–31.
14. Wood MA, Fuller IA. Acute and chronic electrophysiologic changes surrounding radiofrequency lesions. J Cardiovasc Electrophysiol 2002; 13:56–61.
15. Perez FJ, Wood MA, Schubert CM. Effects of gap geometry on conduction through discontinuous radiofrequency lesions. Circulation 2006;113:1723–9.
16. Weerasooriya R, Khairy P, Litalien J, et al. Catheter ablation for atrial fibrillation: are results maintained at 5 years of follow-up? J Am Coll Cardiol 2011;57:160–6.
17. Ouyang F, Tilz R, Chun J, et al. Long-term results of catheter ablation in paroxysmal atrial fibrillation: lessons from a 5-year follow-up. Circulation 2010;122:2368–77.

Key Words: ablation ■ atrial fibrillation ■ histology.