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ORIGINAL ARTICLE

Histological assessment of transmurality after repeated radiofrequency ablation of the left atrial wall

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Abstract
Objective Radiofrequency ablation (RFA) makes the Cox-Maze procedure less complicated but cannot always achieve transmural lesions. In this study, we assessed whether repeated application of RFA could improve histological transmurality even in thick lesions.
Methods Left atrial appendages (LAA) amputated from 20 consecutive patients (age 68 ± 9 years, 9 males) who underwent the Cox-Maze procedure using bipolar RFA devices were studied. Three different segments in the amputated LAA were ablated once, twice, or three times. Thereafter, cross sections made along each of the ablation lines were histologically assessed. Transmurality, which was defined as completion of transmural fibrotic changes, and wall thickness were investigated at an average of 3 different sites in each section.
Results A total of 177 sites were investigated and divided into groups with single, double, and triple RFA treatments for 56, 61, and 60 lesions, respectively. Transmural lesions were observed in 25 (45 %), 27 (44 %), and 41 (68 %) lesions, respectively ($P = 0.011$). The transmurality was 100 % for all lesions with a thickness of 1.0 mm or less. In the thicker lesions (>1.0 mm), however, wall thickness impaired transmurality, though triple RFA was associated with significantly higher transmurality than single and double RFA ($P = 0.005$).
Conclusions Triple repetition of RFA was associated with higher transmurality of lesions than double RFA, especially for thicker lesions. Increasing the number of repetitions could improve the success rate of the Cox-Maze procedure using RFA devices.

Keywords Atrial fibrillation · Surgical ablation · Radiofrequency ablation · Maze procedure

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Introduction

Radiofrequency ablation (RFA) is commonly used in surgical ablation for atrial fibrillation because it can reduce the operative risk via its procedural simplicity [1]. However, the success rate of surgical RFA is not as good as that of the cut-and-sew technique, possibly because RFA cannot always achieve transmural lesions [2, 3]. RFA causes thermal necrosis on the atrial wall and results in electrophysiological conduction block if the necrosis occurs transmurally [4]. Thus, the transmurality of the RFA lesion may be a keystone in terms of the long-term success of Cox-Maze procedure using RFA. Although the development of bipolar RFA devices has improved transmurality [5], failure can occur, especially in thicker lesions, because the wall thickness of the atrium also affects the transmurality [4, 6]. Thus, repeated ablation has been recommended for further transmurality [5, 7]. However, we sometimes experience incomplete conduction block, which can be proved intraoperatively using temporary pacing, even after a second ablation [8]. We hypothesized that repetitive RFA that is applied considering the risk of incomplete transmurality such as the thickness of lesions could improve the transmurality and success rate of Cox-Maze procedure using RFA devices. Therefore, in this study, we assessed the efficacy of repetition of RFA in terms of the histological transmurality of lesions taking into account the wall thickness.

Patients and methods

Study population

Twenty consecutive patients who underwent the Cox-Maze procedure for chronic atrial fibrillation using a bipolar RFA clamp with amputation of the left atrial appendage (LAA) between January 2010 and May 2011 were studied. Their mean age was 68 ± 9 years and 9 (45 %) were male. Two different bipolar RFA devices were used: a temperature-controlled device (Cobra Bipolar, Estech Inc., Camino Ramon, CA, USA, $n = 10$) and an impedance-controlled device (Isolator Synergy, Atricure Inc., Cincinnati, OH, USA, $n = 10$). Table 1 shows the patients' characteristics. All the patients underwent the Cox-Maze procedure as a concomitant procedure. Surgery was performed through a median sternotomy using standard cardiopulmonary bypass. The Cox-Maze procedure was performed during cardiac arrest. During the procedure, the LAA was amputated in all the cases followed by sutural closure of its orifice. All the procedures were finished uneventfully in all the patients.

Table 1 Patients' characteristics

Age, years	68 ± 9
Male, *n* (%)	9 (45)
Chronic atrial fibrillation, *n* (%)	20 (100)
Type of radiofrequency device, *n* (%)	
Temperature-controlled	10 (50)
Impedance-controlled	10 (50)
Main operative procedures, *n* (%)	
Mitral valve repair	14 (70)
Mitral valve replacement	4 (20)
Atrial septal defect closure	1 (5)
Pericardiectomy	1 (5)

Study protocol

The transmurality of bipolar RFA lesions was assessed using the amputated LAA. RFA was applied in accordance with the instructions for each device. In brief, temperature-controlled devices deliver RF energy to achieve and maintain a target tissue temperature of >50 °C for 30 s. Power output is automatically adjusted to accommodate various wall thicknesses. In contrast, in impedance-controlled devices, the amount of RF energy is determined depending on tissue impedance. Energy delivery changes throughout the ablation cycle as tissue impedance changes. The ablation is discontinued when the tissue impedance decreases sufficiently and reaches a stable plateau. Three different segments in the LAA were ablated once, twice, or three times (Fig. 1). Before the application of RFA, the LAA was prepared so that there were both thick (double layer) and thin (single layer) lesions in each of the ablation lines to ensure that each lesion had a wide thickness range. After the ablation lines were created, the LAA was fixed by immersion in 10 % formalin solution and embedded in paraffin. Thereafter, serial transverse sections of the LAA that were made along each of the ablation lines were histologically assessed. The transmurality and wall thickness of each RFA lesion were investigated at an average of 3.0 different sites in each section. The sites of investigation were arranged to include thick, medium, and thin lesions evenly in each section. The lesion was defined as transmural if degenerative changes were seen transmurally in the sections with Masson-Trichrome stain (Fig. 2). A cross section that intersected 3 different ablation lines was also investigated and the wall thickness before RFA was estimated by comparing the thicknesses of the ablated lesion and neighboring intact lesion (Fig. 1). The study protocol was approved by the institutional ethical committee and the procedures were performed in accordance with institutional guidelines. Written informed consent was obtained from all patients.

Statistical analysis

All continuous data are expressed as mean ± standard deviation and categorical data are shown as frequency and percentage. Comparisons of transmurality among different groups were conducted using the Chi-square test or Fisher's exact test as appropriate. The relationships between wall thickness in the lesions after RFA and their neighboring sites were estimated using linear regression analysis. A two-tailed $P < 0.05$ was defined as statistically significant in all the tests. All analyses were performed using IBM SPSS Statistics (version 20, IBM Corporation, Somers, NY, USA).

Results

A total of 177 sites were investigated in 60 sections and divided into single, double, and triple RFA lesions for 56, 61, and 60 lesions, respectively. No fracture or perforation of the tissue was observed in any lesion. Preexisting histological abnormalities such as fibrosis and fatty infiltration were observed in almost all sections without ablation. The RFA-induced histological changes occured differently among the lesions. Some lesions came from the both epi- and endocardium (Fig. 2b), whereas others were unilateral (Fig. 2c) or only in the mid-part of the atrial wall. Figure 3 shows the transmurality of the lesions with each number of repetitions of RFA. Transmural lesions were observed in

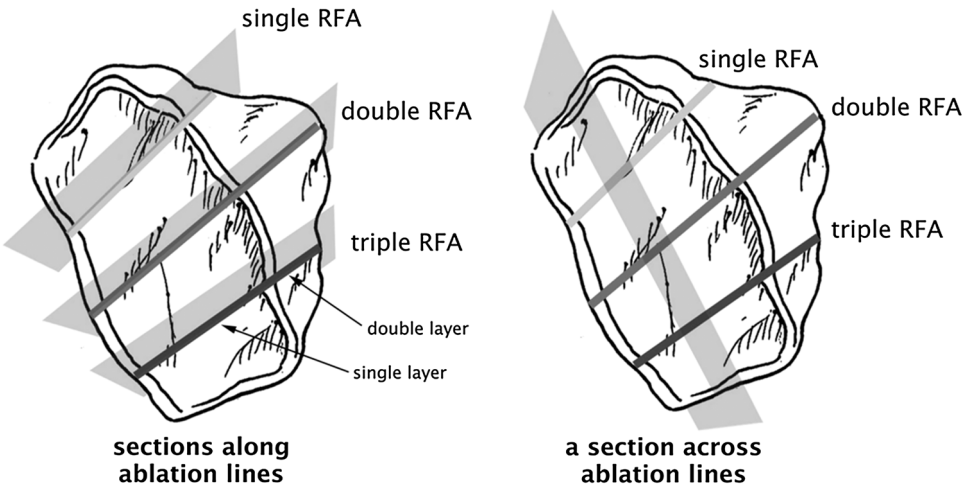

Fig. 1 Schematics of sections in the amputated left atrial appendage used for histological investigations

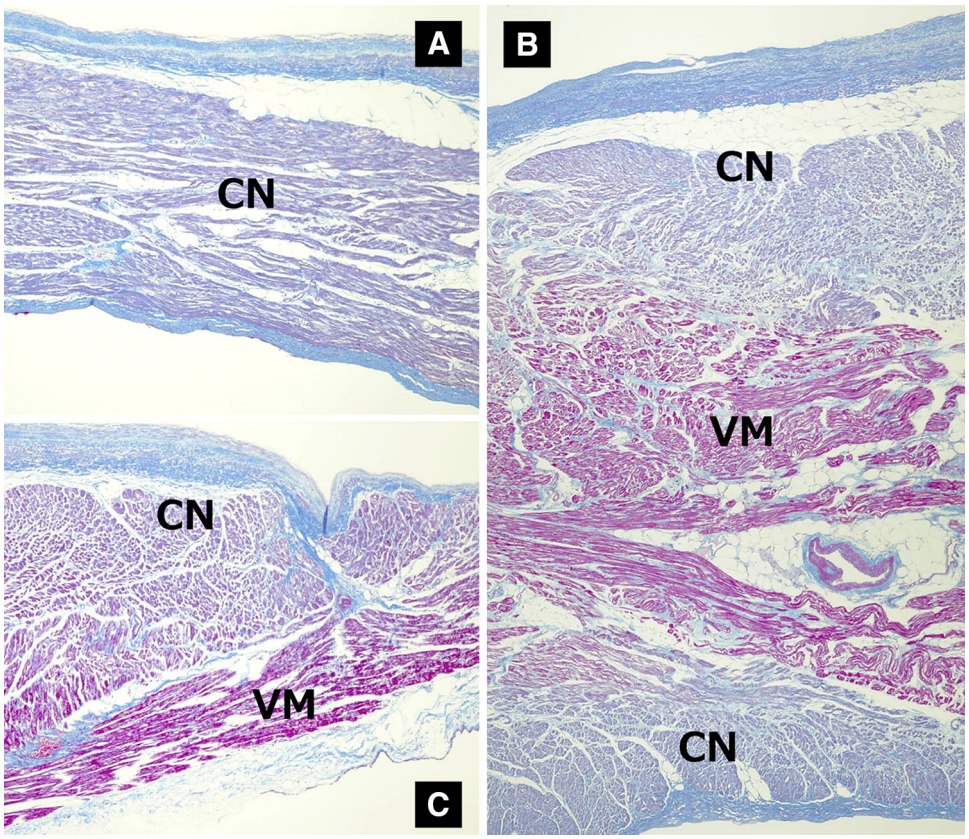

Fig. 2 Histological assessment of transmurality in sections with Masson-Trichrome stain: transmural lesion (**a**) and nontransmural lesion (**b**, **c**). The ablated area with coagulation necrosis (CN) is stained *blue* and the *red* stain represents viable myocardium (VM)

25 (45 %), 27 (44 %), and 41 (68 %) lesions after single, double, and triple RFA, respectively ($P = 0.011$). Triple RFA lesions exhibited significantly greater transmurality than single or double lesions with both the temperature- and impedance-controlled devices. Figure 4 shows the relationships among the number of repetitions, wall thickness, and transmurality. Increased wall thickness affected the transmurality of the lesions. All the thin (1.0 mm or less) lesions were completely transmural. In the thicker lesions (>1.0 mm), however, transmurality was significantly higher for triple RFA lesions than for the others ($P = 0.005$). In addition, as the lesions become thicker, the difference of transmurality between triple and other RFA lesions becomes more evident. In contrast, the transmurality in the thicker lesions (>1.0 mm) was not significantly different between the temperature- and impedance-controlled devices for single, double, and triple RFA ($P = 0.26$, $P = 0.79$, and $P = 0.27$, respectively). Figure 5 shows the relationships between the wall thickness of the lesion after RFA and its neighboring sites,

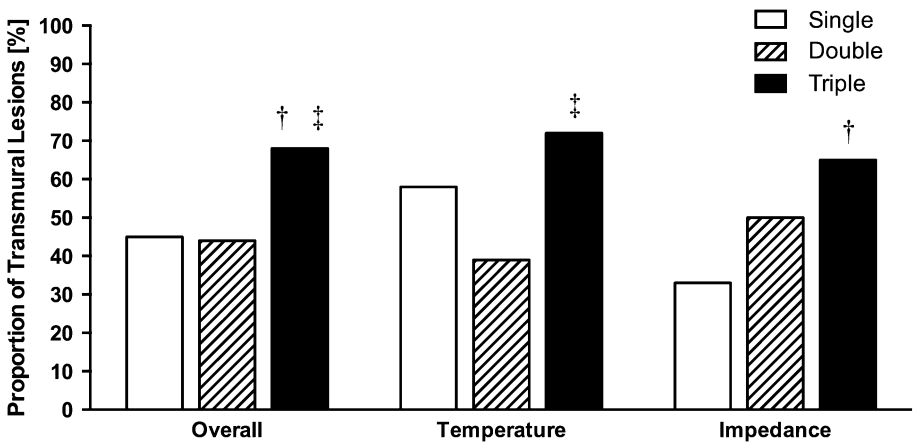

Fig. 3 Transmurality for each number of repetitions of ablation. $^{\dagger}P < 0.05$ compared with single RFA, $^{\ddagger}P < 0.05$ compared with double RFA. *RFA* radiofrequency ablation

which may represent lesions before RFA. Because all lesions with post-RFA wall thickness of 1.0 mm or less were transmural, these relationships suggested that transmurality could be expected for the lesions with a pre-RFA wall thickness of 2.0, 2.3, and 2.7 mm or less for single, double, and triple RFA, respectively. In contrast, the reduction rate of wall thickness after RFA was significantly different between temperature- and impedance-controlled devices, especially for multiple RFA lesions (single: 39 ± 21 vs. 43 ± 18 %, $P = 0.66$; double: 45 ± 17 vs. 64 ± 13 %, $P = 0.017$; triple: 57 ± 11 vs. 70 ± 11 %, $P = 0.028$).

Discussion

In this study, we demonstrated that triple RFA was associated with higher transmurality than single or double RFA, especially for thicker lesions. Increasing the number of repetitions of RFA improved transmurality, especially for the thicker lesions, and could result in better success rates for the Cox-Maze procedure using RFA devices.

Unlike the classical cut-and-sew technique, RFA requires transmural necrosis to achieve electrophysiological conduction block [4]. Transmurality in the lesions created by unipolar RFA was impaired as the wall thickness increased. Transmurality in the ostium of the pulmonary vein (thickness = 1–3 mm) and in the left atrial isthmus (thickness = 4–10 mm) was achieved in 96 and 14 % of the lesions after unipolar RFA, respectively. In contrast, development of a bipolar clamp improved the transmurality. In experimental studies using animals, a single bipolar RFA clamp could achieve transmurality in 100 % of the lesions where the maximum wall thickness was 5–10 mm [9, 10]. In the clinical setting, however, a single bipolar RFA clamp frequently fails to create transmural lesions and repetition of RFA has been recommended to ensure transmurality [5, 7]. Nevertheless, a double bipolar RFA clamp also failed in the thicker lesions or at the folds of the atrial wall [6, 8]. Because nontransmural lesions may result in the failure of the Maze procedure [11], thick lesions should be carefully treated. To investigate the efficacy of bipolar clamps in such conditions, we evaluated lesions with a range of wall thicknesses by ablating both single- and double-layered atrial walls simultaneously, and we measured the wall thickness of the lesions away from the gap between single- and double-layer lesions because the transmurality could be affected at the gap. As a result, we found that wall thickness impaired the transmurality of RFA lesions, though the triply applied bipolar RFA clamp was associated with higher transmurality than double RFA, especially for the thicker lesions.

Triphenyltetrazolium chloride (TTC) staining is the standard method for infarct size delineation in experimental cardiology because delayed expansion of transmural lesions may have to be taken into account [12]. However, in our protocol using resected tissues, the potential infarct size could be underestimated because proper infarct delineation by TTC staining requires a certain reperfusion time to wash out NADH from the infarcted area [13, 14]. Although the assessment using Masson-Trichrome stain could also underestimate the extent of transmurality after RFA, our results were consistent with previous clinical studies. Stulak et al. [3] reported that the results of the Cox-Maze procedure with RFA did not reach those of cut-and-sew even with double RFA. In contrast, a recent study reported that the Cox-Maze procedure with RFA resulted in approximately 90 % freedom from atrial fibrillation [15]. They applied bipolar RFA 2–3 times until conduction block was detected by temporal pacing. Thus, repeated ablation, sometimes more than twice, could contribute to further transmurality for thick lesions such as those in the atrial isthmus or the double-layered wall of the pulmonary vein in the isolation technique, and improvement of the results of Cox-Maze procedure using RFA devices.

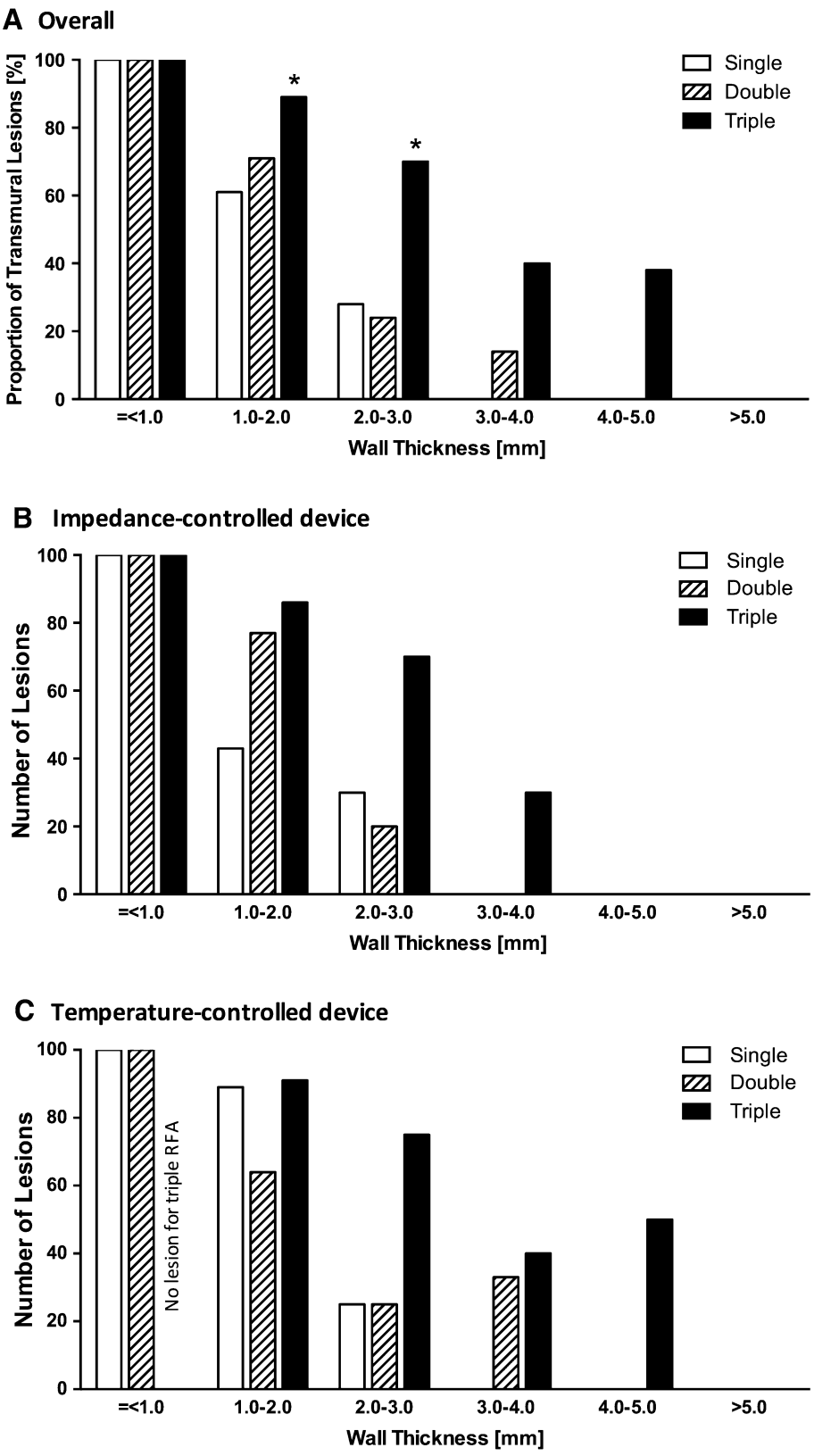

Fig. 4 Relationships among number of repetitions, wall thickness, and transmurality for all lesions (**a**), lesions with impedance-controlled devices (**b**), and lesions with temperature-controlled devices (**c**). *RFA* radiofrequency ablation

In this study, we used two different types of devices: temperature- and impedance-controlled clamps. We found no remarkable difference in the histological findings between the lesions made with these devices. In contrast, with the impedance-controlled device there was greater reduction of wall thickness after RFA clamping than with

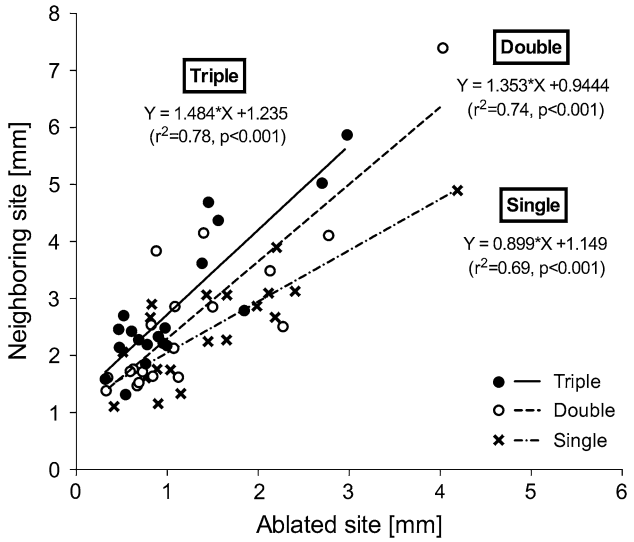

Fig. 5 Relationships of wall thickness between ablated site and its neighboring site for single, double, and triple radiofrequency ablation

the temperature-controlled device, although the transmurality in the lesions with both devices was affected similarly by post-RFA wall thickness. This suggested that an impedance-controlled device could be more useful for preoperatively thicker lesions than a temperature-controlled device. Further study will be required to assess the device-specific differences.

Conclusions

Triply repeated RFA was associated with a higher success rate of transmural lesions than single or double RFA, especially for thicker lesions. We recommend repeated application of RFA more than twice for lesions where an incomplete transmural lesion can be expected after standard single or double RFA considering the wall thickness of the lesions.

Conflict of interest All authors have no conflict of interest.

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