Effect of Left Atrial Ablation Process and Strategy on Microemboli Formation During Irrigated Radiofrequency Catheter Ablation in an In Vivo Model

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Background—Formation of microemboli during catheter ablation has been suggested as a cause for asymptomatic cerebral emboli. However, it is unknown which part of the process and ablation setting/strategy is most strongly related to this occurrence.

Methods and Results—A total of 27 pigs were used. Catheter/sheath manipulations in left atrium were performed in 25 of 27 pigs outfitted with microemboli monitoring systems. Ablations using open-irrigated radiofrequency catheters were performed in 18 of 25 pigs. Two of 27 pigs did not undergo left atrial procedures and were injected with microembolic materials in the carotid artery to serve as positive controls. In total, 334 sheath/catheter manipulations (transseptal puncture, sheath flushing, catheter insertion, pulmonary vein venography, and sheath exchange) and 333 radiofrequency applications (power setting, 30/50 W; point-by-point/drag ablations) were analyzed. High microbubble volume in the extracorporeal circulation loop and a high number of microembolic signals in carotid artery were observed during sheath/catheter manipulations especially in saline/contrast injections at fast speed and ablations with steam pop. Fast sheath flushing produced significantly higher microbubble volume than slow sheath flushing (median, 12200 versus 121 nL; P<0.0001). A total of 44 of 126 (35%) blood filters in the circulation loop showed microparticles (thrombus/coagulum and tissue). Most of them were seen after radiofrequency application especially in 50-W ablations, drag ablations, and steam pop. Brain magnetic resonance imaging showed positive-embolic lesions in control pigs.

Conclusions—Formation of microbubbles was the greatest during fast saline/contrast injections and steam pops, whereas high-power radiofrequency applications, drag ablations, and steam pops produced most of the microparticles.

Key Words: catheter ablation | embolism | magnetic resonance imaging | microbubbles | pulmonary veins

Catheter ablation for drug-refractory atrial fibrillation has become an established therapy. Open-irrigated radiofrequency catheters were designed to reduce electrode–endothelium interface temperature, thereby decreasing thrombus formation.1 A recent systematic review shows that the incidence of peri-procedural symptomatic stroke or transient ischemic attack related to thrombus was 0.2% to 0.3%.2 However, other studies have revealed the incidence of asymptomatic cerebral emboli to be between 2% and 40% in patients.3–6 Formation of microbubbles and microparticles during catheter procedure may lead to cerebral injury. Haines et al7 reported microemboli formation using multielectrode-phased radiofrequency and irrigated radiofrequency catheters in a swine model. In a different study using a canine model, they reported that microbubbles and microparticles were thought to be the source of these asymptomatic cerebral emboli.8 However, it is not known which part of the procedure during left atrial (LA) ablation is most strongly related to microbubble and microparticle formation. In addition, there is limited characterization of microemboli formation using open-irrigated radiofrequency catheters.

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The aims of this study were, therefore, to (1) investigate the timing and quantification of microbubble/microparticle formation during LA catheter procedures, using multiple real-time monitoring systems in an in vivo swine model; (2) examine microbubble/microparticle characteristics during each ablation process; (3) compare microbubble/microparticle formation between different sheath/catheter manipulations and ablation settings/strategies; (4) investigate the relationship between microbubbles monitored in the LA using intracardiac echocardiography (ICE) and peripheral microembolic signals monitored; (5) examine the relationship between microbubble/microparticle formation and cerebral embolic lesions using magnetic resonance imaging (MRI); and (6) thereby explain previous findings of asymptomatic cerebral embolic events.

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WHAT IS KNOWN

- Asymptomatic cerebral emboli have occurred in 2% to 40% of patients after catheter ablation for atrial fibrillation.
- Formation of microbubbles and microparticles during catheter procedure are thought to be the source of the cerebral emboli.

WHAT THE STUDY ADDS

- Frequent microbubble formation occurred with sheath/catheter manipulations, especially in rapid saline/contrast injections.
- High power, drag ablation, and steam pop resulted in an excessive amount of microparticle formation.

Methods

Study Design

Acute and chronic studies were performed in a total of 27 pigs using a protocol aimed at simulating a clinical LA ablation procedure. For the initial acute studies (19 pigs), during various processes associated with an LA procedure, microbubble/microparticle formation was monitored by real-time monitoring systems. In the following 8 chronically studied pigs (n=2, positive control; n=6, ablation), brain MRI was performed in addition to the catheter procedures with real-time microembol monitoring.

General Methods

The protocol was approved by the Mayo Foundation Institutional Animal Care and Use Committee. Swine (70–110 kg) were anesthetized using telazol, ketamine, and xylazine, intubated, and maintained on 1% and 3% isoflurane. Surface ECG and SpO₂ were continuously monitored.

A 19-Fr cannula was placed into the left femoral artery and a 20-Fr cannula into the left femoral vein for placement of an extracorporeal circulation loop system. A 12-Fr sheath was placed in the right external jugular vein. An 8-Fr sheath was placed in the right femoral artery for blood pressure monitoring. An 8.5-Fr SL1 sheath (St. Jude Medical, St. Paul, MN) was inserted into the right femoral vein. An unfractionated heparin bolus was administered after deployment of the large cannulas. The activated clotting time (ACT) was measured every 30 minutes, and heparin bolus was administered after deployment of the large cannulas.

Transseptal Puncture and Sheath Exchange

Transseptal puncture was performed using a transseptal needle (BRK; St. Jude Medical, St. Paul, MN) through the SL1 sheath under ICE and fluoroscopic guidance. After confirmation of entry into the fossa ovalis, the transseptal needle was advanced through the septum. Passing through the fossa ovalis, 5 mL saline was injected to confirm the position of the needle tip on ICE. Subsequently, the SL1 sheath was advanced into the LA, and it was exchanged over a 0.032-inch J-wire to an 8.5-Fr Agilis sheath (St. Jude Medical, St. Paul, MN) or a 12-Fr Zº Flex-270 sheath (Boston Scientific, Marlborough, MA).

Catheter Insertion in the LA

Three types of catheters (7-Fr ablation catheters, 7-Fr, 15-mm Lasso mapping catheter [Biosense Webster Inc, Diamond Bar, CA], and 6-Fr angiographic catheter) were inserted into the LA through the sheath.

Microemboli Monitoring

Figure 1 depicts the real-time monitoring systems used to detect microbubble/microparticle formation.

Extracorporeal Circulation Loop System

An extracorporeal loop system, equipped with an ultrasound Doppler system (BC100: GAMPT Ultrasonic Solutions, Merseburg, Germany) for detection of gaseous microbubbles, was used. In addition, microparticles were detected using an incorporated INLINE filter. Further details of the system settings are described in the Data Supplement. A shunt flow rate of >1.5 L/min was used as an indication of good blood flow over the course of the procedure. The cardiac output of the pigs in this study was assumed to be 4–5 L/min. Therefore, the loop flow comprised ~30% to 40% of the total cardiac output. Microbubble data are expressed as microbubble volume (nL) in each sheath/catheter manipulation and each radiofrequency application. Microparticles were analyzed after formaldehyde fixation.

Doppler Ultrasound System in Left Common Carotid Artery

Monitoring the common carotid artery for embolic signals has been reported previously. The left common carotid artery was continuously monitored using a Neurovision Doppler ultrasound system (Multigon Industries Inc, Yonkers, NY). Details of the system settings are described in the Data Supplement. Microembolic signals were identified according to advocated criteria. The number of microembolic signals was manually counted in each sheath/catheter manipulation and each radiofrequency application.

Intracardiac Echocardiography

A 10-Fr, 5.5- to 10-MHz ultrasound catheter probe (ICE; Acuson-Siemens, Mountain View, CA) was used to monitor/record LA microbubble formation. Before the start of each radiofrequency ablation, microbubble formation from the irrigation flow was monitored from the catheter tip as a baseline image. Microbubble formation during radiofrequency ablation was compared with baseline microbubbles. Microbubble formation was divided into 3 types of microbubble densities; no bubble or isolated bubbles were categorized as “few”, those with continuous but nondere appearance as “moderate”, and those with continuous and dense appearance as “shower”. Microbubble findings were analyzed offline to correlate microbubble volume in the circulation loop and the number of microembolic signals in the carotid artery. LA microbubble formation was analyzed in each sheath/catheter manipulation and each radiofrequency application.

![Image of real-time microemboli monitoring system](http://example.com/image1.png)

**Figure 1. Setup of the real-time microemboli monitoring system.** Intracardiac echocardiography (ICE) was placed for monitoring of microbubbles in the left atrium. The left common carotid artery was used to monitor microemboli signals for the brain by ultrasound Doppler. The extracorporeal circulation loop was placed as a shunt between the femoral artery and vein, which was equipped with a blood filter to assess microparticle formation. Two ultrasonic bubble counter heads were placed on the loop to monitor microbubble volume.
The analysis was done in a blinded fashion with respect to which intervention was tested.

Specific Methods—Interventional Steps

Sheath Flush
Sheaths were flushed in the LA after initial insertion, before catheter/wire insertion, after sheath exchange, and after pulmonary vein (PV) venography. Sheath flushing in the LA was performed in the following 2 ways: (1) 5 to 10 mL blood was withdrawn from the sheath and discarded and then 8 mL saline solution was injected with a new syringe; and (2) 1 mL blood was withdrawn from the sheath and then 8 mL saline solution was injected without discarding the blood. A previous study showed that injection speed affected microembolic formation during cerebral angiography. Therefore, sheath flushing speed was randomized for high volume rate (fast speed) at 8 mL/1 s and low volume rate (slow speed) at 8 mL/5 s. Throughout the procedure, the sheath was continuously irrigated with heparinized saline. To examine whether an iatrogenic atrial septal defect after transseptal puncture affected the microembolism in the LA, sheath flushing with saline was also performed in the right atrium.

PV Venography
PV venography was performed using a 6-Fr angiographic catheter. For placement of the angiographic catheter in the PV, a test injection (2 mL) of contrast medium (iohexol, 350 mgI/mL; Novaplus) was performed through the angiographic catheter. Subsequently, 8 mL of contrast medium was injected for PV venography.

Radiofrequency Ablation
In the acute study, radiofrequency ablation was performed in 12 of 19 pigs in the LA and at the LA–PV junction using either the 3.5-mm tip ThermoCool (TC) catheter (6 pigs; Biosense Webster, Inc., Diamond Bar, CA) or the 4-mm tip Blazer Open-Irrigated (BOI) catheter (6 pigs; Boston Scientific, Marlborough, MA). In the chronic study, radiofrequency ablation was performed using BOI catheter (6 pigs). Ablation power setting was 30 W or 50 W. Saline irrigation flow was set at 2 mL/min during mapping, 20 mL/min during ablation with 30 W, and 30 mL/min during ablation with 50 W. Point-by-point ablation was applied at each point for 30 or 60 s. Drag ablation was performed using a 4-mm step, slow drag of the catheter tip each 30 s over 240 s duration along the LA roof and over the mitral isthmus. A steam pop was defined as an audible pop associated with a sudden spike in impedance on recorded graphs during ablation.

The blood filter in the circulation loop was examined/replaced at the following time points: (1) after all sheath/catheter manipulations, (2) after 30-W, 30-s point-by-point ablations, (3) after 30-W, 60-s point-by-point ablations, (4) after 30-W drag ablations, (5) after 50-W, 30-s point-by-point ablations, (6) after 50-W, 60-s point-by-point ablations, and (7) after 50-W drag ablation.

Postprocedure MRI Scan
For the 8 pigs in the chronic study, 6 of 8 pigs underwent MRI scanning 2 to 4 days after completion of the LA ablation procedure. In 2 of 8 pigs used as positive controls without any procedures in LA, microembolic material was injected into the carotid artery to examine whether brain embolic lesions could be created and detected by MRI. Detailed settings of MRI are described in the Data Supplement. Diffusion-weighted imaging, gradient recalled echo T2*, and T2 fluid-attenuated inversion recovery were used. Embolic materials were created as described.

Coagulum particles were created from blood that was heated and dried. The size range of coagulum particles was 0.1 to 1.5 mm, a similar size to the microparticles captured on the blood filter. Microbubbles were made by mixing 0.5 mL air, 2.5 mL blood, 1.25 mL contrast medium, and 1.25 mL saline. Three injections (each >30 particles or 0.5 mL air) were made into the right and left carotid arteries. One of the 2 pigs that underwent microemboli injection died just after the procedure.

Pathology
In acute studies, pigs were euthanized just after the catheter procedure. For the chronic studies, pigs were euthanized after the MRI scanning. Triphenyl tetrazolium chloride was used to delineate ablation lesions. Heart, lungs, trachea, and esophagus were removed en block with the pericardium intact. Ablation lesions were grossly assessed. Cerebrum, cerebellum, brain stem, kidneys, liver, and spleen were dissected. The brain, kidneys, and spleen were sectioned into 5-mm thick slices. Tissues were fixed in formaldehyde and stained with hematoxylin-eosin and Masson trichrome.

Statistical Analysis
Statistical analysis was performed using SAS 9.4 (SAS Institute, Cary, NC). Continuous variables were examined with the Shapiro–Wilk test for normality. All continuous variables had some evidence of a non-normal distribution, so the data were presented as median with the quartiles (median [quartiles]). Because multiple measurements had been obtained per animal, the data were analyzed using generalized estimated equation models with an exchangeable correlation structure. A rank transformation of the data was applied to provide more of a normal distribution for the comparison. Categorical variables were also compared using generalized estimated equation models and an exchangeable correlation structure. The P values are 2-sided. A P value of >0.05 was considered to indicate statistical significance.

Results
Total Process
Table 1 shows all interventional steps and their respective number in this study. In total, 334 sheath/catheter manipulations and 333 radiofrequency applications were analyzed. Figure 2 shows ICE-monitored microbubble formation in the LA. Figure 3 shows the microbubble volume in the extracorporeal circulation loop and the number of microembolic signals in the carotid artery. Figure 4 shows microparticle formation in the blood filter in the circulation loop. Of 126 filters examined, 44 filters showed microparticles. The median number of microparticles that was seen in all filters during 1 procedure was 5 (4–6) per pig.

Sheath/Catheter Manipulations
Transseptal Puncture and Sheath Exchange
ICE showed moderate or shower microbubble formation in the LA in 50% of transseptal punctures (Figure 2). The median microbubble volume in the circulation loop was 2060 (274–6830) nL, which was the third highest volume for all interventional steps (Figure 3A). The number of microembolic signals seen in the carotid artery was 17 (11–32; Figure 3B). Most microembolic signals were recorded during saline injection for confirmation of transseptal needle position in the LA.

During sheath exchanges, the median microbubble volume was 811 (518–1390) nL and the number of microembolic signals was 16 (15–17). Most microembolic signals were seen during atrial septum crossing.

Sheath Flush and PV Venography
Shower type microbubbles in the LA were seen by ICE in 70% of sheath flushed at high volume rate (fast speed; Figure 2). Fast sheath flush also produced the second highest microbubble volume (12 200 [4065–22 325] nL) and microembolic signals (26 [12–46]) of all procedures (Figure 3).

In contrast, in most sheath flushed at low volume rate (slow speed), ICE showed few microbubbles in the LA, and no shower microbubble formations were seen. The median
Microemboli Formation During LA Ablation Procedure

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Microbubble volume (121 [30–409] nL) and number of microembolic signals (3 [1–5]) were also low. During saline flushing in the right atrium, microbubbles in the LA were rarely observed. The microbubble volume was 50 (1–130) nL, and the number of microembolic signals was 0 (0–1).

During contrast test injections and PV venographies, moderate or shower microbubbles in the LA were commonly seen (Figure 2). The median microbubble volume was 1740 (815–5285) nL in contrast test injections and 1640 (724–4715) nL in PV venography. The median number of microembolic signals was 10 (8–15) and 11 (9–18), respectively (Figure 3).

Catheter Insertion

Few or moderate microbubbles in the LA were seen during catheter insertion via the sheath (Figure 2). The microbubble volume was 381 (169–792) nL, and the number of microembolic signals was 7 (3–9; Figure 3).

Microparticle Formation After Sheath/Catheter Manipulations

Two of 21 (10%) filters were positive for microparticles after all sheath/catheter manipulations (Figure 4). The diameter of microparticles was 0.5 mm (0.3–0.9). Histologically, these microparticles were identified as thrombus.

Open-Irrigated Radiofrequency Ablation

Table 1 shows the total number of each type of radiofrequency application (300 point-by-point ablations and 33 drag ablations) and the occurrence of steam pop in both acute and chronic animals. Steam pops were seen in 43 of 333 (13%) of all radiofrequency applications.

![Figure 2. Intracardiac echocardiography monitoring of bubble formation in the left atrium (LA). The graph shows the percentage of bubble type in each interventional step. Drag indicates drag ablation; point-by-point, point-by-point ablation; PV, pulmonary vein; and RA, right atrium.](image-url)
Thirty-Watt Point-by-Point and Drag Ablation Without Steam Pop

Few microbubbles were seen in the LA during 83% of 30-W point-by-point ablations (Figure 2). The microbubble volume was the lowest (36 [2–122] nL) in all procedures. The number of microembolic signals was 2 (0–5; Figure 3). Figure 4 shows the incidence of microparticles in the filter during radiofrequency ablation. Microparticles were seen in 3 of 36 (8%) filters with 30-W point-by-point ablation. The diameter of microparticles was 0.7 mm (0.5–1.0). Histologically, all microparticles were thrombus/coagulum. In contrast, moderate bubbles were seen in 79% of 30-W drag ablations. The microbubble volume was 148 (70–524) nL, and the number of microembolic signals was 14 (5–24). Four of 14 (29%) filters showed thrombus/coagulum formation after 30-W drag ablation with a microparticle diameter of 0.6 mm (0.5–0.7).

Fifty-Watt Point-by-Point and Drag Ablation Without Steam Pop

ICE showed few or moderate microbubbles during 50-W point-by-point ablation without steam pop (Figure 2). The microbubble volume was 62 (3–186) nL. The number of microembolic signals was 4 (1–9; Figure 3). Microparticles (thrombus/coagulum) were seen in 4 of 17 (24%) filters (Figure 4) with a diameter of 0.7 mm (0.5–0.9).

ICE showed moderate or shower microbubbles in the LA during 50-W drag ablation. The microbubble volume (998 [768–6000] nL) and the number of microembolic signals (17 [6–45]) were also higher than other radiofrequency applications. Microparticles were seen in 60% of filters with a diameter of 0.9 mm (0.7–1.1). In addition to thrombus/coagulum, yellow or white microparticles, consisting of endothelial tissue and myocardium, were also seen.

Figure 3. Microbubble volume in the circulation loop and number of microembolic signals in the left common carotid artery during each interventional process are shown. A, Microbubble volume. B, The number of microembolic signals. Drag indicates drag ablation; point-by-point, point-by-point ablation; PV, pulmonary vein; and RA, right atrium.

Figure 4. Microparticles in the blood filter. A, Coagulum on filter after 50-W point-by-point ablation. B, Tissue piece on filter after 50-W drag ablation with steam pop. C, Incidence of the filters with microparticles in each interventional step.
Ablations With Steam Pop
ICE showed shower microbubbles in 80% of ablations with steam pops (Figure 2). The microbubble volume was 15,600 (4560–34,000) nL, and the number of microembolic signals was 52 (32–112). These were the highest numbers (Figure 3) seen in all interventional procedures. Over 80% of filters showed microparticles (Figure 4) with a diameter of 1.0 mm (0.5–2.3). Histopathologically, microparticles were tissue and thrombus/coagulum.

Comparison of Different Open-Irrigated Catheters
Table 2 shows a comparison of ablation data between the TC and BOI catheters. The electrode tip temperature was significantly lower for BOI than TC (P<0.001). BOI produced less microbubble volume than TC at 30 W/50 W (each P<0.001). The number of microembolic signals at 30 W was significantly lower in BOI (P<0.001). The number of steam pops and incidence of microparticles were not significantly different between the 2 catheters. Histologically, ablation lesion size was not significantly different.

Ablation Lesion Characteristics
Figure 5 depicts various ablation lesions in the LA and at the LA–PV junctions. The diameter of all point-by-point ablation lesions at the endocardial surface was 7.4 mm (6.4–8.5; Figure 5A). The length of drag ablation lesions was 30.0 mm (29.8–30.2; Figure 5B). Steam pop lesions were marked by destructive crater formation of the endocardial surface with a size of 14.5 mm (13.0–18.9; Figure 5C and 5D). In some cases, coagulum was attached to the ablative lesion (Figure 5D).

Comparison of Sheath/Catheter Manipulations
Figure 6 displays a comparison of microbubble volume and number of microembolic signals during different sheath/catheter manipulations in the LA. When comparing sheath flushing at fast speed to flushing at slow speed, fast sheath flushing produced a significantly higher microbubble volume and number of microembolic signals (each P<0.0001; Figure 6A). When comparing the different ways in saline flushing at fast speed, microbubble volume and number of microembolic signals were a little higher in flushing without discarding blood, but these were not significantly different (Figure 6B). Comparing different catheter insertions into the LA, insertion of a circular mapping catheter tended to produce higher microbubble volume and microembolic signals than the insertion of other catheters; however, there were no significant differences among catheter types (Figure 6C).

Comparison of Ablation Setting/Strategy
Comparing 30-W and 50-W point-by-point ablations (excluding ablations with steam pops), ablations with 50 W produced higher microbubble volume (62 [3–186] versus 36 [2–122] nL; number of microembolic signals (each P<0.0001; Figure 6A). When comparing the different ways in saline flushing at fast speed, microbubble volume and number of microembolic signals were a little higher in flushing without discarding blood, but these were not significantly different (Figure 6B). Comparing different catheter insertions into the LA, insertion of a circular mapping catheter tended to produce higher microbubble volume and microembolic signals than the insertion of other catheters; however, there were no significant differences among catheter types (Figure 6C).

Figure 5. Macroscopic findings of the left atrium (LA) and pulmonary vein (PV) after ablation. A, Point-by-point ablation lesion in the LA and LA–PV junctions. B, Drag ablation lesion from the mitral annulus to pulmonary vein. C and D, Ablation lesion with steam pop (black arrows). White arrows indicate ablation lesions with coagulum formation. LAA indicates left atrial appendage; and MVA, mitral valve annulus.
A higher number of microembolic signals \((4 \pm 1–9)\) versus \(2 \pm 0–5\); \(P<0.001\), and a higher incidence of microparticles in the filter \((24\% \pm 8\%; \ P=0.10); \ Figure 7A\). Figure 7B shows the comparison of the microemboli formation per 60 s between 30-W point-by-point ablation and 30-W drag ablation (excluding ablations with steam pop). Microbubble volume per 60 s during radiofrequency ablations was not significantly different between these 2 groups \((47 \pm 2–125)\) versus \(37 \pm 18–131\) \(\mu L\); \(P=0.49\)). However, the number of microembolic signals per 60 s tended to be higher in drag ablation (point-by-point ablation: \(2 \pm 0–6\) versus drag ablation: \(4 \pm 1–6\); \(P=0.14\)). The incidence of microparticle formation in the filter also tended to be higher in drag ablations (point-by-point ablation: \(17\%, n=17\) versus drag ablation: \(29\%, n=19\); \(P=0.46\)).

**Correlation Between ICE Findings and Peripheral Parameters**

Figure 8 shows the correlation between ICE-monitored microbubbles and peripheral findings. Figure 8A shows the relationship between ICE-monitored LA microbubbles and measured microbubble volume in the circulation loop; the microbubble volume was \(43 \pm 2–185\) \(\mu L\) when few bubbles were observed on ICE. For moderate bubbles, the volume was \(600 \pm 202–2030\) \(\mu L\), and \(16900 \pm 6083–32950\) \(\mu L\) when shower bubbles were seen. Microbubble formation observed on ICE strongly correlated with microbubble volume in the circulation loop. Figure 8B displays the correlation between ICE-monitored LA microbubbles and the number of microembolic signals in the carotid artery. The number of microembolic signals was \(3 \pm 0–5\) when few bubbles were observed on ICE, \(9 \pm 5–17\) in cases of moderate bubbles, and \(36 \pm 16–56\) when shower bubbles were seen. Microbubble formation observed on ICE also correlated with the number of microembolic signals.

**Cerebral Findings and Other Organs**

Brain MRI in the positive-control pig showed multiple subcortical hyperintense lesions on T2 and diffusion-weighted imaging, indicative of embolic lesions. There was no evidence of acute embolic lesions in the brain MRI of the other 6 pigs that underwent the ablation procedure. No embolic lesions were observed macroscopically or microscopically in any part of the brain. Acute embolic lesions were also not identified in the kidney or the spleen of any animals.

**Discussion**

**Major Findings**

Pertinent findings of this study are (1) sheath/catheter manipulation, especially rapid saline/contrast injections, produced

![Figure 6](http://circ.circjour.ahajournals.org/)

Figure 6. A, Comparison between sheath flush at fast speed (8 mL/1 s) and slow speed (8 mL/5 s). B, Comparing sheath flushing at fast speed after discarding blood and flushing at fast speed without discarding blood. C, Comparison between insertion of 3 different catheters.
many microbubbles; (2) microparticles (coagulum, thrombus, and tissue) in the filter were not commonly seen during sheath/catheter manipulations with high-dose anticoagulation strategy (ACT>350 s); (3) most microparticles were seen during point-by-point ablation using 50 W, drag ablations with 30 W or 50 W, and ablations with steam pops; (4) ablations with steam pops showed the highest microbubble volume, microembolic signals, and microparticles; the size of microparticles observed after steam pop was larger than that found in other procedures; and (5) LA microbubbles observed on ICE correlated well with peripheral microbubble volume in the external circulation loop and number of microembolic signals in carotid artery.

Data in the Context of Recent Clinical Studies
Recent clinical studies have shown that the anticoagulant strategy and the energy sources of catheter ablation are major factors contributing to asymptomatic cerebral embolism. Gaita et al reported that asymptomatic cerebral embolic lesions were observed in 14% of patients after atrial fibrillation ablation using open-irrigated radiofrequency catheters. In that study, the mean ACT during the procedure was 281±34 s, which was lower than that in this study. In addition, unfractionated heparin was given after transseptal puncture. In contrast, Di Biase et al investigated the incidence of asymptomatic cerebral embolism in patients undergoing radiofrequency ablation without warfarin discontinuation and receiving heparin bolus before transseptal puncture (ACT>300 s). This significantly reduced the occurrence of asymptomatic cerebral embolic lesions (2%). Comparing different energy sources of ablation, multielectrode-phased radiofrequency ablation showed higher incidence of asymptomatic cerebral emboli than open-irrigated radiofrequency ablation and cryoballoon ablation. Delivery of bipolar energy between adjacent electrodes especially in overlapping proximal and distal electrodes was found to lead to higher energy delivery and overheating of the tissue and blood, which resulted in microemboli formation. This mechanism may be similar to tissue and blood overheating by ablation with high-power settings or drag ablation using open-irrigated radiofrequency catheters, which resulted in high volume of microbubble and thrombus/coagulum formation in this present study.

Characterization of Microbubbles in Catheter Interventions
This study is the first investigation that showed the source of microbubbles during important interventional steps performed in the LA. Wood et al characterized microbubbles during radiofrequency application as indication for excessive tissue heating and as being composed of steam released from the ablated tissue. For monitoring of these, the bubble counter system in this study has been found to reliably identify gaseous microbubbles in an extracorporeal circulation loop in basic and clinical investigations. Given the strong correlation between LA microbubbles observed on ICE during radiofrequency ablation and sheath/catheter flushes to peripherally detected microbubble volumes, this study suggests that sheath/catheter procedural steps are a source of microbubbles independent of radiofrequency application.
This study revealed a relatively low incidence of microparticle (clot/thrombus) formation during sheath/catheter manipulations compared with radiofrequency applications. Previous studies showed the risk of thrombus formation during sheath/catheter manipulations in the LA.\textsuperscript{15,19} In this study, large thrombus on the sheath/catheter was not visible on ICE. This might be because of the high-dose anticoagulation therapy used in this study. Heparin was administered just after cannula placement in the femoral artery/vein, which might have reduced thrombus formation. Single transseptal puncture and sheath insertion in this study might also have reduced the risk of thrombus formation. However, some blood filters showed small thrombi that might have been released from the sheath.
The circular mapping catheters tended to produce higher microbubble volume and numbers of microembolic signals than other linear catheters. The shape of the circular mapping catheter is more complex compared with linear catheters, which might result in the introduction of more thrombus or air. In addition, our laboratory has reported greater thrombogenicity of the circular mapping catheter during PV isolation.19

Ablation Procedure

Point-by-Point Ablation

Our data suggest that the risk of microbubble and microparticle formation was minimized during low-power, point-to-point ablations using an open-irrigated radiofrequency catheter. Our laboratory previously reported microbubble formation using closed-loop, cooled-tip catheters.20 In this study, microbubble formation was not commonly seen during point-by-point ablation without a steam pop, reflecting a stronger cooling effect at the catheter–tissue interface with an open-irrigated catheter. However, ablation with a high-power setting (50 W) induced excessive heating of tissue and blood with a higher risk of coagulum formation. Comparing the 2 different open-irrigated catheters used in this study, the BOI catheter showed lower electrode temperature, microbubble volume, and the number of microembolic signals. Both catheters have 6 irrigation holes; however, the BOI catheter has dual cooling chambers, and the direction of irrigation flow is more proximal to the tip than the TC catheter, which might lead to more uniform cooling of the electrode tip.

Drag Ablation

Drag ablation tended to cause more microemboli than point-by-point ablation. Although the tip of the catheter was moved 4 mm each 30 s during drag ablation, some ablation lesions overlapped. The tissue of overlapped areas was heated for longer times, possibly resulting in overheating of the tissue, and may have caused higher thrombus/coagulum and microbubble formation. In addition, drag ablation might release more thrombus/coagulum adhering to the endocardium.

Ablations With Steam Pops

This study confirmed that steam pops produce high-volume microemboli. Steam pops are caused by excessive intramyocardial heating to temperatures of >100°C with overheating resulting in tissue water boiling and steam formation, which could erupt through the endocardial surface.21 The size of microparticles varied greatly (quartiles, 0.5–2.3 mm). This reflects that, in addition to overheating of tissue and blood, steam pops result in tissue pieces being carried away in the bloodstream.

Cranial MRI Findings

The contribution and clinical relevance of microemboli formation during ablation to embolic cerebral lesions could not be resolved in this study. No evidence of acute cerebral embolism was seen in any pigs that underwent catheter ablation although microparticles were captured in the blood filter. First, the anatomy of the cerebral circulation in pigs is different from human; pigs have large anastomosing of vessels, a so-called rete mirabile, which could have worked as a filter for microemboli and thus could have affected outcomes of MRI and cerebral tissue evaluations. Second, the number of microparticles formed during LA intervention might have been too small to create detectable brain embolic lesions in the number of chronic animals used in this study. Third, tiny embolic infarcts may have been below the spatial resolution of the MRI scan that was 2 mm×2 mm×3 mm.

The pathological significance of microbubbles has been studied by Haines et al22 who reported that injection of agitated saline–contrast–blood mixture (1.5 mL air) in the carotid artery was able to create brain embolic lesions in the canine model. On the other hand, Helps et al23 reported a similar volume of 25 μL (25,000 nL) of air microbubbles as created by interventions in our present study, for causing deterioration of brain function and blood flow in a rabbit model.

Although other procedures are different from catheter ablation, during on-pump coronary artery bypass grafting, a total microbubble volume of 4 to 20 μL was also observed during the procedure using the same bubble counter system.19 It remains unclear how much microbubble volume is needed to cause brain tissue embolic events.

Limitations

First, anatomy and tissue characteristics in the pig model are not the same as in humans. However, all interventional steps in this model were carried out in a similar fashion as in the clinical laboratory and thus are thought to be comparable with clinically performed LA ablation. Secondly, this is an in vivo study using open-irrigated radiofrequency catheters. Therefore, contact force/interface area between the catheter tip and tissue, orientation of the catheter tip, and blood flow around the catheter might have been different for each radiofrequency application. This might have affected microemboli formation. Third, the animal model in this study was not an atrial fibrillation model, reflecting a different thrombogenic state. The number of animals that underwent MRI scanning in this study was relatively low, making underdetection of cerebral lesions caused by microbubbles and microparticles (type II error) possible. Fourth, the filter was not checked after each sheath/catheter manipulation; therefore, we could not determine microparticle formation during each sheath/catheter manipulation. Fifth, the brain and other organs were sectioned into 5-mm thick slices. A slice could be seen as a shallow depth of 10 to 30 μm. Therefore, 0.5% to 1.0% of the total volume of the brain and other organs were examined. Thus, we cannot exclude that small embolic lesions were not present. Sixth, there was no adjustment for multiple comparisons across the many variables because of the nature of the study.

Clinical Implications

Recently, catheter ablation is increasingly offered to patients with atrial fibrillation. This study suggests that several processes in the LA procedure carry the risk of microemboli formations. Saline/contrast injection at fast speed produced high microbubble volume and number of microembolic signals; therefore injections and sheath flushes should be done at slow speed, significantly reducing microbubble/microemboli volume. Intervention with complex-shaped catheters should be performed with caution, and sheaths should be well-aspirated...
and flushed after removal. Point-by-point radiofrequency ablation with high-power setting and drag ablation should also be done with caution. It is well known that cardiac perforation and tamponade occur after steam pops. However, greater attention should be given to thromboembolism after steam pop occurrence. On the basis of our data, intraprocedural monitoring of LA with ICE could be useful to assess real-time microembolic burden.

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SUPPLEMENTAL MATERIAL

1. The arterial part of this system consisted of a 19 Fr cannula inserted into the femoral artery that diverted blood through the tubing, equipped with a pre-filter ultrasonic bubble counter, blood filter (73 μm polyester filtration membrane [Sefar-Petex #07-73-40 membrane]) for detection of microparticles, and a post-filter ultrasonic bubble counter for detection of microbubbles. Blood was returned to the animal through the venous return side via a 20 Fr cannula placed in the femoral vein. A flow meter (Bio Medicus, Minneapolis, MN, USA) was also placed in the circulation loop. A shunt flow rate > 1.5 L/min was used as an indication of good blood flow over the course of the procedure.

2. A 2 MHz Doppler probe was placed on the left neck and positioned with an adjustable headband. The Doppler angle was aligned to the blood flow, so that a strong signal could be obtained. The multi-depth Doppler window function was used. Consistently, strong baseline Doppler signals were recorded at a depth of 35-55 mm. The sample volume was 10 mm.

3. MR scans were performed with a 1.5-T scanner (HD 16.0_V02, GE Medical Systems) in 2 pigs and a 3-T scanner (HD 16.0_V02, GE Medical Systems) in 5 pigs. A 6-channel animal head coil made at Mayo Clinic was used to optimize signal to noise ratio. A b-value was 1000 ms. The following MRI-sequences were used: axial T2, axial and coronal diffusion–weighted imaging (DWI), axial gradient recalled echo T2*, and axial T2 fluid-attenuated inversion recovery (FLAIR). The parameters of the sequences were as follows. Axial T2: TR 4400 ms, TE 119 ms, slice thickness 3.0mm, field of view 14.0 cm, and matrix 224×224. DWI: TR 5500, TE minimum, slice thickness 3.0 mm, field of view 24 cm, matrix 140×140 (axial), 140×84 (coronal). Axial gradient recalled echo T2*: TR 1600 ms, TE 26 ms, flip angle 20°, slice thickness 3.0mm, field of view 14.0 cm, and matrix 224×128. A board-certified neuroradiologist analyzed all MRI scans.