Atrial fibrillation (AF) is not a life-threatening disease by itself; however, it worsens heart failure and an uncontrolled rate can result in tachycardia-induced myopathy. But foremost, it quintuples the incidence of fatal strokes. An appropriate anticoagulation therapy reduces the stroke risk, although not to zero. The indication for prevention was decided by simple risk scores, such as the CHADS2 and CHA2DS2-VASc scores. However, the net clinical benefit of warfarin administration is not apparent in non-valvular AF patients with a low CHADS2 score because of the major bleeding risk associated with the lifelong intake of anticoagulants compared with the low risk of an embolism. In contrast, patients with a high CHADS2 score actually receive benefit from anticoagulant administration, but usually the risk of major bleeding is also high according to the HAS-BLED score. The use of anticoagulants also remains controversial for high CHADS2 patients with a history of bleeding complications, dual antiplatelet therapy, and a potential risk of bleeding such as aneurysms. The more recent emergence of several new oral anticoagulants has made introducing anticoagulants easier compared with warfarin, but the contradiction between the necessity of anticoagulation and the risk of bleeding remains. An appropriate prevention strategy is thus still needed to reduce the incidence of AF diagnosis poststroke.

Background—Approaches for closing the left atrial appendage (LAA) have been developed for stroke prevention. However, the prevailing maneuvers require an open-chest surgery, intravascular access, or transseptal puncture. We evaluated the feasibility and safety of pericardial endoscopy-guided LAA ligation in a canine model.

Methods and Results—We used a total of 8 canines and computed tomography was performed before the procedures. After a double percutaneous pericardiocentesis, a transurethral rigid endoscope was inserted into the pericardial space. The ENDOLOOP ligature was advanced to the ostium of the LAA by counter pulling the tip of the LAA with forceps. After confirming the positioning guided by transesophageal echocardiography, the ligature was securely tightened. Acute success was evaluated by transesophageal echocardiography and chronic success was evaluated by blood testing, computed tomography, and transesophageal echocardiography. The LAA ligation was safely achieved in all canines without major complications. One month after the ligation, the ligated LAA was replaced by fibrotic tissue, and both the transesophageal echocardiography and computed tomographic images revealed no residual shunt. There was only a localized adhesion of the pericardium, where the original LAA was located, without the need for antibiotic or steroid administration. The postprocedural internal surface of the ligated LAA was smooth by virtue of intimal growth. Blood tests showed a slight elevation of the inflammatory markers, but this normalized spontaneously.

Conclusions—Pericardial endoscopy-guided LAA ligation could provide an alternative, minimally invasive, and feasible solution for LAA closure that does not require vascular access or a transseptal puncture. (Circ Cardiovasc Interv. 2014;7:844-850.)

Key Words: appendage ■ endoscopy ■ ligation
WHAT IS KNOWN
- The left atrial appendage accounts for 90% of the embolic sources in atrial fibrillation.
- Closing the left atrial appendage is a potential strategy for preventing cardioembolic strokes.
- Prevailing strategies for ligating the left atrial appendage require either an open-chest surgery, vascular access, or transseptal puncture.

WHAT THE STUDY ADDS
- A pilot study that demonstrates the feasibility and safety of a pericardial endoscopy-guided left atrial appendage ligation.
- Computed tomography and transesophageal echocardiography demonstrate a successful ligation in all cases.
- No residual shunt or pericardial adhesion was identified 1 month after the procedure.
- A pericardial endoscopy-guided left atrial appendage ligation can be an alternative approach with only pericardial access.

Occlusion Study (LAAOS) showed the safety of LAA ligations in patients undergoing coronary artery bypass grafts. Other techniques have since emerged such as the WATCHMAN, ATRICLIP, and LARIAT, and the evidence of the efficacy techniques have since emerged such as the WATCHMAN, ATRICLIP, and LARIAT, and the evidence of the efficacy of accumulating for these procedures. Because these procedures require either an open-chest surgery or a transseptal puncture under guidance of fluoroscopy, a novel procedure for LAA ligation thus remains sought after.

Previously, we demonstrated the possibility of pericardial endoscopy in the field of arrhythmia treatment. We aimed to apply this technology for closing the LAA under clear visualization without requiring an open-chest surgery, vascular access, or a transseptal puncture. Here, we conducted a preliminary study to evaluate the feasibility of a pericardial endoscopy-guided LAA ligation technique tested in a canine model (see Movie in the Data Supplement).

Methods

All experimental protocols were approved by the institutional ethical committee.

Ligation Procedure

Step 1: Preparation

We used a transurethral rigid endoscope (A22003A, Olympus Medical Systems Corporation, Tokyo, Japan), which we previously showed to be feasible and safe for surgical maneuvers (Figure 1A). This endoscope has a 70-degree squinted and 90-degree visual field that provides easy handling and safety for adequate visualization of the heart in a narrow pericardial space. We prepared handmade sheaths with a 5-mm inner diameter for the camera and 7.5-mm inner diameter for the ligating tools, which were composed of a liguette, LAA forceps, and loop holder. We used the ENDOLOOP ligature (monofilament PDS II Suture, Ethicon Inc, Somerville, NJ), but replaced the plastic strut with a metal spring sheath (NM-101C-0427, Olympus Medical Systems Corporation; Figure 1B and 1). The LAA forceps (Endo Relief, J3101, Hope Denshi Co, Ltd., Kamagaya, Chiba) were originally designed for endoscopic surgery, to grasp objects without inducing any mechanical damage. The loop holder (RGF-3115, Gyrus ACMI Inc, MA) was used only as needed for holding the opposite side of the loop if it was not large enough to bundle all the LAA lobes. All equipment was gas sterilized before being used.

Step 2: Pericardial Access

Canines were sedated with an infusion of 0.5 mg/kg of pentobarbital. The animals were intubated and ventilated with room air using a constant-volume cycled respirator (Model SN-480-3, Shinano Inc, Tokyo, Japan). General anesthesia was maintained with 1.5% halothane in the supine position. A normal saline infusion was continued via the forearm to compensate for the body fluid loss. The ECG and pulse oximetry were continuously monitored during all procedures. A transesophageal echocardiography (TEE) probe (HD11 XE, Philips Electronics Japan, Ltd, Tokyo, Japan) was inserted through the esophagus to view the ostium of the LAA.

After local anesthesia of the epigastric fossa, a double percutaneous pericardiocentesis was performed by a modified Seldinger technique with an 18-gauge epidural anesthesia needle filled with a contrast medium under the guidance of fluoroscopy and echocardiography. The puncture holes of the pericardium were located on the anterior wall of the heart to ensure easy access to the LAA. The Camera Sheath and Tool Sheath were gently advanced and placed in the pericardial space side by side. The guidewire (Radifocus Guidewire M, Terumo Corporation, Tokyo, Japan) used for pericardiocentesis remained inside the pericardial space to avoid the unintentional removal of the sheaths. A right side-lying position was preferred when it was difficult to observe the LAA as described in our previous article.

Step 3: Ligation

The LAA forceps were placed inside the ligature loop and inserted through the Tool Sheath to gently grasp the LAA and pull it toward the sheath (Figure 2A and 2B). The ligature was advanced and the loop was placed at the ostium of the LAA by pushing the metal spring sheath toward the ostium (Figure 2C–2E). Once the position of the ligature was confirmed by TEE, the end of the loop was pulled upward to secure the knot (Figure 2F). The LAA forceps were then released and pulled away, followed by the metal spring sheath (Figure 2G). The end of the loop was held by the loop holder to keep widening the circle of the loop if loop positioning was difficult or when the loop was too small to bundle all the LAA lobes. Scissors (A64820A, Olympus Medical Systems Corporation) were inserted through the Tool Sheath and the extra ligature was cut and withdrawn (Figure 2H). The camera and both sheaths were then removed from the epicardial space (Figure 2I), and the puncture holes were sutured with a 3-0 Vicryl suture (Ethicon Inc).

Study Protocol

A total of 8 canines each weighing 20.3±0.7 kg were used in this study. Six canines were euthanized 1 month after the procedure for the chronic evaluation and 2 canines were euthanized immediately after the procedure for the acute evaluation. A macroscopic observation after the animals were euthanized was performed for all evaluations. The chronic evaluation included blood sampling, computed tomography (CT), and TEE before and after the procedure.

Blood Sampling

Blood samples were taken from the animals before, and at 2, 3, 4, and 9 days after the procedure (SRL Inc, Tokyo, Japan) for the following tests: complete blood cell count, coagulation and fibrinolysis status, kidney function, liver function, and creatine kinase (CK), isozyme of CK (CK-2), lactate dehydrogenase, C-reactive protein, procalcitonin, amyloid A, haptoglobin, human atrial natriuretic polypeptide, and brain natriuretic peptide levels.
Computed Tomography
CT imaging was performed before the procedure and euthanasia. Canines were sedated with 0.5 mg/kg of pentobarbital. A total of 1 mg of propranolol was administered when the heart rate was above 130 beats/min. Canines were fixed to the table in the supine position and intubated to control the respiratory movements, and contrast medium was administered via the forearm. ECG-gated CT imaging (Aquilion CXL, Toshiba Medical Systems, Otawara, Japan) was initiated 5 and 22 seconds after the infusion of 30 cc of contrast medium. Three-dimensional images with an automated cardiac phase selection were reconstructed and measured using specialized software (AZE VirtualPlace, AZE, Ltd., Tokyo, Japan).

Transesophageal Echocardiography
During the procedure, TEE was used for positioning the ligature, measuring the LAA size, and evaluating the residual shunt. For the chronic evaluation, the residual shunt status was observed before euthanasia.

Statistical Methods
Continuous variables were expressed as median±standard deviation. A Wilcoxon signed-rank test was used to compare the numeric blood testing data. A probability value of <0.05 was considered statistically significant.

Results
Efficacy
The LAA ligation was safely completed in all canines. The acute evaluation of 2 canines showed no dislodgement or loosening of the ligature. Positioning the ligature at the ostium of the LAA was initially confirmed by TEE (Figure 3A and 3B) with an average diameter of 1.3±0.1 cm (Table). By gradually tightening the loop, the width of the ostium was narrowed (Figure 3C), and the position was easily realigned by pulling the LAA forceps in front and counterpushing the ligature forceps. The metal spring sheath instead of the plastic strut

Figure 1. Tool configuration. The device configuration of the camera and tools (A) by a schema (B) and image (C). The camera and tools for ligating the left atrial appendage (LAA), consisting of the ligature (ENDOLOOP), forceps, and loop holder, were inserted through 2 handmade sheaths. The ligature was placed at the ostium of the LAA by pulling the LAA and pushing the ligature. The black bar indicates 1 cm.

Figure 2. Maneuver of the left atrial appendage (LAA) ligation. Step 1, grasping the LAA (A, B); Step 2, advancing the ligature to the ostium (C-E); Step 3, tightening the knot (F); Step 4, releasing and cutting the extra loop, such that the LAA is securely ligated at the ostium without any major complications (G-I). The procedural time was <4 minutes.
of the ligature worked well to deliver the appropriate power directly toward the LAA ostium; the metal spring sheath was able to let much power dissipate by bending (Figure 2E and 2F). The flow of the LAA ostium stopped completely when the loop was slowly tightened and then the knot fastened for a few seconds to complete the ligation (Figure 3D and 3E). There was no bleeding or mechanical damage by grasping or ligating the LAA (Figure 2G and 2H), and no loosening of the knot after a single ligation procedure (Figure 3F). The color of the LAA turned grey immediately after the ligation, and the systolic movement of the LAA had ceased shortly after the ligation (Figure 2G–2I). A Swartz sheath (SR0, St. Jude Medical Inc, St. Paul, MN) was useful for suctioning the extra fluid such as contrast medium. We used an optional loop holder to hold the opposite side of the loop and enlarge the loop in 2 of the canines, in which the LAA was relatively large and the puncture holes of the pericardium were positioned far toward the front to allow a right-side approach to the LAA.

The internal surface of the LAA orifice was examined before (Figure 4A), immediately after (Figure 4B) and 1 month after the procedure (Figure 4C). Interestingly, the internal surface of the ligated ostium was remarkably smooth, being covered by intimal growth without thrombus formation or a residual cavity. Compared with the normal LAA (Figure 4D), the ligated LAA had completely shrunken and was replaced by hard fibrotic tissue (Figure 4E). A pericardial adhesion was marked only around the ligated LAA site, but elsewhere, the tissue was patent with no pericarditis, hemorrhagic pericardial effusion, or mechanical damage (Figure 4F). The CT and 3-dimensional reconstructed images were compared before (Figure 5A–5C) and 1 month after (Figure 5D–5F) the procedure. The CT before the ligation revealed LAAs with an average volume of 6.1±0.8 mL and an ostial area of 2.5±0.4 cm² (Table). The CT after the ligation clearly revealed a missing LAA with no residual flow or residual LAA lobes. The TEE imaging before being euthanized also exhibited no LAA cavity, demonstrating a complete and successful LAA ligation with the ligature placed precisely in the orifice of the LAA.

Safety
There were no major complications throughout the experiment. The acute evaluation revealed no bleeding or mechanical damage caused by grasping or ligating the LAA. The heart rhythm was maintained as sinus rhythm with a heart rate from 100 to 140 beats/min throughout the procedure, except for paroxysmal AF that was induced by mechanical stimulation when ligating the LAAs and terminated spontaneously within a few seconds without any hemodynamic deterioration. The oximetry was stable during the entire procedure. The blood tests revealed an increased white blood cell count (day 3 versus baseline, 11,905±2334 versus 8050±340 cells/µL, P=0.068), CK level (151.0±9.9 versus 82.0±8.5 IU/L, P=0.068), CK-2 level (4.1±1.3 versus 7.8±2.0 IU/L, P=0.068), haptoglobin level (67.0±32.6 versus 180.0±19.0 IU/L, P=0.068), and d-dimer level (2.1±0.8 versus 0.1±0.0 IU/L, P=0.068) at day 3, but these levels normalized within a week. There was no significant difference in the levels of the hemoglobin, C-reactive protein, procalcitonin, amyloid A, human atrial natriuretic polypeptide, brain natriuretic peptide,
or lactate dehydrogenase, nor were there any adverse effects on the kidney or liver function. There was no systemic infection, problems with the puncture sites, or global pericarditis, and no postprocedural requirement for systemic antibiotics or intrapericardial steroids.

Noninvasiveness
As shown in Figure 2, the time required for ligating the LAA was <4 minutes once the camera and tools were in place. The total procedure time from the sterilization to suturing of the puncture site was 24±5 minutes with 3±1 minute of fluoroscopy. The puncture holes were <5 mm and 7.5 mm wide, respectively, and the pericardial holes were closed for precaution sake.

Discussion
We performed a pilot study to demonstrate the feasibility and safety of a novel LAA ligation maneuver by using pericardial endoscopy, CT, and TEE in a canine model. The pericardial endoscopy-guided LAA ligation could become an alternative approach without requiring an intravascular approach, transseptal puncture, or open-chest surgery.

Comparison With Prevailing Methods
Ligating the LAA was a simple and classic method previously performed by open-chest surgery15,16 and natural orifice transluminal endoscopic surgery.23 Our pericardial endoscopy-guided LAA ligation was less invasively performed without an open-chest surgery but only with percutaneous pericardiocentesis in all cases. Because neither the intravascular approach nor transseptal puncture was required, unlike the WATCHMAN17 and LARIAT,19 we did not administer procedural anticoagulation, which might be required in humans when considering the risk of embolization. The clear visualization was enough for us to immediately identify bleeding and cardiac rupture. Using the 2 types of ligatures at the same time might be useful for increasing the long-term success rate.

Figure 4. Macroscopic images. Visualization of the internal surface of the left atrial appendage (LAA) ostium before (A), immediately after (B), and 1 month after (C) the procedure. The deep cavity of the LAA was covered up by intimal proliferation. The heart surface showed a pericardial adhesion only around the original LAA (F), which was shrunk and replaced by fibrotic tissue (E), compared with the normal LAA (D).

Figure 5. Computed tomography (CT). CT images before (A–C) and 1 month after (D–F) the procedure are shown. Two-dimensional images (A, D) and 3-dimensional reconstructed images in the superior (B, E) and left lateral (C, F) planes revealed complete ligation of the left atrial appendage (LAA) ostium.
because the double ligation technique has been correlated with lower rates of mortality.\textsuperscript{24}

In terms of disadvantages, the exclusion criteria for the pericardial access were as for other methods with pericardial access, ie, patients with a history of pericarditis, cardiac surgery, recent myocardial infarction, or thoracic radiation. The difficulties in ligating a larger LAA, lobed LAA, or diseased LAA, and the LAA position should be evaluated in further study. For coordinating the anatomic variations, the puncture site of the pericardium was important. The access of the tools was targeted more toward the anterior-left, and the camera was aimed more toward the anterior-middle region of the heart. A controllable tip of the camera might be useful for horizontally dilated hearts in humans, which would be designed for further study. Because there are many types of LAAs classified by their size, angle, and number of lobes,\textsuperscript{25} the tools and maneuvers should be modified for any evolving varieties.

### Long-Term Outcome

Regardless of the methods used for occluding the LAA, the presence of a residual shunt in the very late phase is always a consideration. A previous report showed that an incomplete LAA ligation during mitral valve surgery was identified in 36% patients, whereas a spontaneous echo contrast or thrombus was identified in 50%, and thromboembolic events in 22% patients.\textsuperscript{26} These risks of late leakage with a partial reconstitution of the LAA ligation might induce a contradiction for the following anticoagulation\textsuperscript{27} because a residual shunt might end up with a thrombus formation.\textsuperscript{28}

A left atrial clot after a successful LAA ligation even with a LARIAT was also reported and the thrombus at the site of the closure could arise from a prothrombotic surface induced by the trauma from pulling on the balloon-tipped catheter.\textsuperscript{29} Thus, antplatelet or anticoagulant therapy is advisable until the closure could arise from a prothrombotic surface induced by the trauma from pulling on the balloon-tipped catheter.\textsuperscript{29} A follow-up TEE can confirm the absence of any thrombi in the prevailing maneuver.\textsuperscript{30} In our pericardial endoscopic approach, there was no residual leak or signs of thrombus formation 1 month after the procedure. A study with a larger number of subjects with a longer observation period should be performed to decide the outcome and necessity for postprocedural anticoagulation.

Although some of the prevailing studies did not clearly show a benefit for appendage occlusion,\textsuperscript{31} it was a fact that the majority of the elderly patients with only a risk of a stroke received insufficient anticoagulation.\textsuperscript{32} The pericardial endoscopy-guided LAA ligation could therefore become an alternative solution in the future by developing a countermeasure for anatomic variations, downsizing, and collecting evidence of the long-term efficacy.

### Limitations

The number of cases was too small to elucidate the characteristics of this procedure. The ligation could be modified, for example, in size and thickness because the human LAA is relatively larger, and the risk of amputating the LAA with a thin ligature was not evaluated. The potential risk of damaging viable atrial tissue by pulling in a diseased human atrium should be evaluated because the procedural safety was measured only in normal tissue. The heart muscle damage was not fully elucidated by the use of human biomarkers in the animal blood sample. The risk of perioperative thromboembolic events also was not evaluated because of an underlying rhythm, and the need for perioperative anticoagulation during AF was difficult to evaluate in the animal model.

### Conclusions

The pericardial endoscopy-guided LAA ligation procedure might become an alternative, minimally invasive, and feasible solution without the need for open-chest surgery, vascular access, or a transseptal puncture.

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### Disclosures

We report grants from Olympus Corporation. We have a patent for the copy-guided LAA ligation could therefore become an alternative solution. The number of cases was too small to elucidate the characteristics of this procedure. The ligation could be modified, for example, in size and thickness because the human LAA is relatively larger, and the risk of amputating the LAA with a thin ligature was not evaluated. The potential risk of damaging viable atrial tissue by pulling in a diseased human atrium should be evaluated because the procedural safety was measured only in normal tissue. The heart muscle damage was not fully elucidated by the use of human biomarkers in the animal blood sample. The risk of perioperative thromboembolic events also was not evaluated because of an underlying rhythm, and the need for perioperative anticoagulation during AF was difficult to evaluate in the animal model.

### References


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