

Relationship between the posterior atrial wall and the esophagus: Esophageal position during atrial fibrillation ablation

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BACKGROUND Atrial fibrillation ablation implies a risk of esophageal thermal injury. Esophageal position can be analyzed with imaging techniques, but evidence for esophageal mobility is inconsistent.

OBJECTIVES The purpose of this study was to analyze esophageal position stability from one procedure to another and during a single procedure.

METHODS Esophageal position was compared in 2 patient groups. First, preprocedural multidetector computerized tomography (MDCT) of first pulmonary vein isolation and redo intervention (redo group) was segmented with ADAS 3DTM to compare the stability of the atrioesophageal isodistance prints. Second, 3 imaging modalities were compared for the same procedure (multimodality group): (1) preprocedural MDCT; (2) intraprocedural fluoroscopy obtained with the transesophageal echocardiographic probe in place with CARTOUNIVUTM; and (3) esophageal fast anatomic map (FAM) at the end of the procedure. Esophageal position correlation between different imaging techniques was computed in MATLAB using semiautomatic segmentation analysis.

Introduction

Pulmonary vein isolation (PVI) can now be considered firstline rhythm control therapy for patients with symptomatic paroxysmal atrial fibrillation (AF) episodes or persistent AF without major risk factors for AF recurrence as an alternative to antiarrhythmic drugs.¹ A PVI approach implies unavoidable ablation lesions on the posterior atrial wall. The left atrial (LA) wall is a thin structure with heterogeneous thickness (range <1 mm to >5 mm), with important inter- and

Address reprint requests and correspondence: Dr Antonio Berruezo, Heart Institute, Teknon Medical Center, C/ Vilana, 12, 08022 Barcelona, Spain. E-mail address: antonio.berruezo@quironsalud.es. **RESULTS** Thirty-five redo patients were analyzed and showed a mean atrioesophageal distance of 1.2 \pm 0.6 mm and a correlation between first and redo procedure esophageal fingerprint of 91% \pm 5%. Only 3 patients (8%) had a clearly different position. The multi-imaging group was composed of 100 patients. Esophageal position correlation between MDCT and CARTOUNIVU was 82% \pm 10%; between MDCT and esophageal FAM was 80% \pm 12%; and between esophageal FAM and CARTOUNIVU was 83% \pm 15%.

CONCLUSION There is high stability of esophageal position between procedures and from the beginning to the end of a procedure. Further research is undergoing to test the clinical utility of the esophageal fingerprinted isodistance map to the posterior atrial wall.

KEYWORDS Atrial fibrillation; Atrial wall thickness; Atrioesophageal fistula; Catheter ablation; Esophageal position

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intrapatient variability.² The esophagus is closely related to the LA posterior wall. This anatomic relationship depends mainly on aortic arch anatomy and location and its influence on esophageal position.³ Due to the proximity between these structures, posterior wall ablation may result in several complications, ranging from asymptomatic esophageal lesions to perforation with atrioesophageal fistula (AEF), which might be lethal.⁴ Other possible complications include damage to periesophageal nervous plexi, which can result in postablation gastrointestinal complications such as gastroparesis, pyloric spasm, and gastroesophageal reflux.⁵ Therefore, a widespread practice is to routinely modify energy delivery settings when ablating on the posterior wall. In addition,

KEY FINDINGS

- There is high stability of the atrioesophageal relationship between procedures even 6 months apart and of the esophageal position from the beginning to the end of an atrial fibrillation ablation procedure.
- The multidetector computerized tomography (MDCT)derived esophageal fingerprinted isodistance map is a new way of depicting the complex relationship between the esophagus and the left atrial posterior wall.
- The fingerprinted map allows for better understanding of the atrioesophageal relationship, which is fundamental for improving procedural safety.

several techniques, such as endoesophageal temperature monitoring probes, have been developed to lower the risk of complications, although randomized data argue against the usefulness of this method to prevent esophageal thermal lesions⁶ and even point to a potential harm.⁷

Contemporary imaging techniques allow for esophageal position identification, potentially offering new methods to prevent esophageal thermal injury. Nevertheless, it has been reported that atrioesophageal interaction could be dynamic due to esophageal mobility, with diverging evidence available to date.

The aims of the present study were (1) to analyze the stability of esophageal position inside the mediastinum, hypothesizing that it does not differ significantly on multidetector computerized tomography (MDCT) from one procedure to another; and (2) to evaluate the correlation in esophageal position with multimodality imaging performed before, during, and after a single LA ablation procedure. This information could help develop safer ablation strategies by avoiding ablation at the sites closest to the esophagus.

Methods

Patient sample

Two patient populations were analyzed: (1) a group of consecutive patients referred for AF redo ablation procedure from November 2018 to November 2019 for whom the esophageal position on MDCT was compared between the first ablation and the redo procedure (Group 1: MDCT); and (2) a group of consecutive patients referred for any AF ablation procedure from March 2020 to November 2020 in whom the esophageal position was obtained by 3 different imaging methods (MDCT, CARTOUNIVU™ module [Biosense Webster, Diamond Bar, CA], and esophageal fast anatomic map [FAM]) and compared between one another (Group 2: Multimodality). Figure 1 shows the study design. Age <18 years, pregnancy, presence of concomitant investigation treatments, patient's refusal to participate in the study, unavailability of preprocedural MDCT, and contraindication or inability to obtain esophageal FAM were exclusion

criteria. Patient populations were different for both groups. The study complied with the Declaration of Helsinki, and the study protocol was approved by the local ethics committee. All participants included in the study provided written informed consent.

Preprocedural MDCT and image processing

In all patients, an MDCT study was performed before the procedure. MDCT was performed using a dual-source SOMATOMTM Definition Flash 128-slice scanner (Siemens Healthineers, Erlangen, Germany) or a RevolutionTM scanner (General Electric Healthcare, Milwaukee, WI). Images were acquired during an inspiratory breathhold using retrospective electrocardiography-gating technique with tube current modulation set between 50% and 100% of the cardiac cycle. Angiographic images were acquired during injection of a 100-mL bolus of iopromide 370 mg I/mL (Ultravist, Bayer Hispania, Barcelona, Spain) at a rate of 3 mL/s. Data were transmitted to the workstation for postprocessing and reconstructed into axial images with slice thickness of 0.625 mm.

MDCT image postprocessing

MDCT digital imaging and communications in medicine (DICOM) images were analyzed using ADAS 3D[™] software (ADAS3D Medical, Barcelona, Spain) to segment the esophageal anatomy and the atrioesophageal fingerprinted isodistance map. The endocardial and epicardial LA wall layers were segmented according to a previously described method.⁸ The esophageal surface layer was defined by manual delineation into 7 ± 3 slices, followed by an interpolation in the missing slices. Then, esophageal position and the distance between the esophagus and the epicardial LA posterior wall and pulmonary veins (PVs) were automatically measured and projected onto the endocardial shell as a color-coded isodistance fingerprinted map, in which red depicts the closest area with an atrioesophageal distance <1 mm, yellow 1.1-2 mm, green 2.1-3 mm, blue 3.1-4 mm, and purple >4 mm. Figure 2 shows the pipeline of the segmentation method resulting in a 3-dimensional (3D) map of the atrioesophageal relationship, which can be projected to the atrial endocardial mesh and imported into the CARTO navigation system (Biosense Webster, Diamond Bar, CA). The esophageal trajectory as related to the atrial posterior wall was classified into 5 categories (left, central, right, left-central, and right-central).

Esophageal visualization with CARTOUNIVU image integration module

At the beginning of the procedure, the fluoroscopic image is centered within the coordinates of the CARTO navigation system by means of a reference ring located underneath the table at the lower part of the chest/upper abdomen. Then, fluoroscopic images with the transesophageal echocardiographic (TEE) probe positioned in the esophagus are



Figure 1 Study group design. **Top:** Group 1 redo: Comparison of atrioesophageal relationship as seen by the esophageal isodistance fingerprinted map, between preprocedural MDCT at first and redo ablations. **Bottom:** Group 2 multimodality: Comparison of 3 different imaging methods for the same procedure. **Left:** Anteroposterior fluoroscopic view with superposed esophageal anatomy segmented from the MDCT and atrial anatomy in glass mode. **Center:** Anteroposterior fluoroscopic view with the transesophageal echocardiographic probe inserted in the esophagus. **Right:** Anteroposterior fluoroscopic view with superposed esophageal fast anatomic map (ESO-FAM) obtained with the ablation catheter. MDCT = multidetector computerized tomography.

recorded in anteroposterior, right anterior oblique 30°, and left anterior oblique 30° fluoroscopic projections. For image acquisition, the TEE operator was asked to advance the probe in a neutral position to a location further than the fluoroscopic position of the LA. The CARTOUNIVU module provides transfer of fixed fluoroscopic images or cine loops to the CARTO3 system, allowing for real-time visualization of intracardiac catheters in the 3D mapping environment on a background of stored fluoroscopic images (Figure 3, section 1). Every capture automatically enters the original and its 180° invert orientation (as a mirror image for a virtual biplane view). Once the fluoroscopic images were acquired, the TEE probe was removed as soon as possible and at the latest before radiofrequency (RF) application.

Catheter ablation

Catheter ablation was performed by the By-LAW method,⁹ with patients under general anesthesia with high-frequency,

low-volume ventilation. Oral anticoagulation was uninterrupted, and periprocedural anticoagulation was performed to achieve an activated clotting time >300 seconds. All procedures were performed with a single venous femoral access and a near-zero fluoroscopy protocol,¹⁰ which is the usual local protocol except for atypical atrial flutters requiring a reference catheter (right atrial quadripolar; St Jude Medical, Inc., St. Paul, MN). Transseptal puncture was guided by TEE. Then, FAM of the entire LA anatomy and the PVs was acquired with the ablation catheter to integrate it with the LA wall thickness map within the spatial reference coordinates of the CARTO system.

Acute PVI was confirmed after first pass with the standard local single catheter method demonstrating entry block with the absence of PV potentials inside the vein with the ablation catheter placed sequentially in each segment inside the circumferential PV line, and exit block by proving absence of conduction when pacing from inside the circumferential PV line, at each segment sequentially.⁸



Figure 2 Segmentation pipeline of the fingerprinted atrioesophageal isodistance map. **1:** Raw MDCT DICOM images were imported into ADAS 3D for segmentation. **2:** Complete 3-dimensional (3D) reconstruction of the esophageal and atrial anatomy was obtained. **3:** The atrioesophageal fingerprinted isodistance map was projected onto the atrial endocardial surface (atrioesophageal distance: red < 1 mm; *yellow* 1–2 mm; *green* 2.1–3 mm; *blue* 3.1–4 mm; *purple* >4.1 mm). MDCT = multidetector computerized tomography.

Esophageal FAM acquisition

Esophageal geometry was acquired at the end of the procedure with the ablation catheter (ThermoCool SmartTouch or Navistar ThermoCool, Biosense Webster). The operator introduced the catheter into the esophagus with the aid of the anesthetist. The catheter was set to a neutral curve position and gently advanced into the esophagus as far as the lower margin of the LA as seen in the CARTO matrix. Esophageal anatomy was reconstructed by slightly curving the catheter tip with rotational movements (Figure 3, section 1).

Statistical analysis and esophageal position comparison

All applicable statistical tests are 2-sided and performed using a 5% significance level. Continuous variables are given as mean ± SD or median (range or interquartile range [IQR] if data were skewed) if not normally distributed. To compare means of 2 variables, the Student t test, Mann-Whitney U test, or analysis of variance was used, as appropriate. Categorical variables are given as total number (percentage) and were compared between groups using the χ^2 or Fisher exact test. Statistical analysis was performed using IBM SPSS Statistics for Macintosh, Version 25.0 (Released 2017, IBM Corp., Armonk, NY) and customized code for the MATLAB statistics toolbox (MATLAB R2010a, The Math-Works, Inc., Natick, MA). For group 1 (redo), spatial correlation between the 2 MDCT-derived esophageal isodistance fingerprinted maps was calculated using MATLAB. The esophageal fingerprint was calculated over the LA anatomy of the first procedure. Both esophageal fingerprinted isodistance maps were projected over the same LA anatomy for computing the correlation value. Correlation was calculated

between the whole fingerprinted surfaces (Figure 4). For group 2 (multimodality), image correlation between fluoroscopy, FAM, and MDCT was computed in MATLAB using semiautomatic segmentation analysis. Each image technique was either imported and merged (CT) or acquired (UNIVU and FAM) within the spatial reference coordinates of the CARTO system (Figure 3, section 3). After the procedure, the CARTO reference matrix was exported to MATLAB, and the positions as observed by the 3 methods were compared to one another with specifically developed code. A correlation between each pair was obtained in the coronal plane (anteroposterior view). This correlation was expressed as a percentage. Global image correlation was computed as the mean correlation between the 3 modalities was computed.

Results Patient population

Baseline characteristics of the 2 groups are summarized in Table 1. The MDCT group was composed of 39 patients: mean age 61 ± 10 years; 26 (67%) male; mean left ventricular ejection fraction 59% \pm 6%; mean LA diameter 44 \pm 4 mm; mean body mass index 29 \pm 6 kg/m²; and median time since first ablation (and therefore between MDCT acquisitions) 6 months (IQR 3–9).

Group 2 was composed of 100 patients: mean age 61 ± 10 years; 17 (65%) male; mean left ventricular ejection fraction 56% \pm 7%; mean LA diameter 39 \pm 6 mm; and mean body mass index 27 \pm 5 kg/m². No patient was excluded because of inability to perform CT; 1 patient was excluded from group 2 because of an esophageal diverticulum that contraindicated



Figure 3 Esophageal position acquisition methods. 1: Left: Three-dimensional MDCT-derived esophageal anatomy with color-coded atrioesophageal distance gradient (red < 1mm; yellow 1-2 mm; green 2.1-3 mm; blue 3.1-4 mm; purple > 4.1 mm). Center: UNIVU anteroposterior acquisition with the transesophageal echocardiographic probe in place. **Right:** Esophageal fast anatomic map (FAM) obtained with the ablation catheter. 2: Image integration into the navigator. Merging of the atrial FAM with the MDCT-derived left atrial wall thickness map using the CARTOMERGE module. 3: Mean image correlation between methods. MDCT = multidetector computerized tomography.

esophageal FAM. MDCT was performed within 48 hours preceding the intervention. Image segmentation included not only the esophageal anatomy and the atrioesophageal fingerprint but also LA wall thickness information.⁸ Time for UNIVU acquisition was 1.5 ± 1 minutes and for esophageal FAM acquisition was 3 ± 1.5 minutes.

Atrioesophageal distance and position measurements in the MDCT group

Mean atrioesophageal distance was 1.2 ± 0.6 mm. Total fingerprinted area (up to 4-mm distance) was 13.2 ± 5.2 cm² before first ablation and 13.5 ± 5.0 cm² before the redo procedure. The closest fingerprinted area (red <1-mm distance to the LA epicardial wall) was 8.1 ± 2.3 cm² before the first ablation and 8.3 ± 2.5 cm² before the redo procedure (P = .34). The esophageal transversal width at the level of the PVs was 27.2 ± 4.3 mm before first ablation and 27.4 ± 4.4 mm before the redo procedure (P = .37). The esophageal position as related to the atrial posterior wall was left for 20 patients (56%); central for 6 patients (18%); right for 3 patients (9%); left-central for 4 patients (11%); and rightcentral for 2 patients(3%). There was a 91% \pm 5% correlation on the esophageal fingerprint position between the first procedure and the redo procedure MDCT. In 3 cases (8%), the position was clearly different, with a correlation of only 40% \pm 22% (Figure 4).

Atrioesophageal distance and position measurements on the multimodality group

Procedural time was 55 (IQR 50–61) minutes. Mean atrioesophageal fingerprinted distance was 1.3 ± 0.5 mm. Esophageal position as related to the atrial posterior wall was left for 55 patients(55%), central for 23 patients(23%), right for 9 patients (9%), left-central for 8 patients (8%), and right-central for 5 patients (5%). The correlation between MDCT and CARTOUNIVU was $82\% \pm 10\%$; between MDCT and esophageal FAM was $80\% \pm 12\%$; and between



Figure 4 Group 1 (MDCT) results of esophageal fingerprinted isodistance map correlation. **Top:** Case with low correlation. Note the esophageal fingerprint contour in *blue* for the first ablation and in *red* for the redo procedure. **Bottom:** Case with high correlation of esophageal fingerprint between first and redo procedures (95% image correlation). MDCT = multidetector computerized tomography.

esophageal FAM and CARTOUNIVU $83\% \pm 15\%$. The results are shown in Figure 5.

There was an important discordance between periprocedural esophageal position in 8 patients (8%), with mean MDCT to CARTOUNIVU correlation of $52\% \pm 8\%$.

Discussion Main findings

The main findings of the present study are as follows (1) The esophageal fingerprint is a novel 3D method of depicting the relationship between the esophagus and the LA posterior wall derived from the MDCT. (2) There is high temporal stability of the esophageal position between procedures separated by several months. (3) Multiple imaging techniques are available periprocedurally and can be used to evaluate esophageal position. (4) These imaging techniques show high stability of the esophageal position during the procedure in patients under general anesthesia.

Esophageal fingerprint

The esophageal fingerprinted isodistance map is a new way of depicting the complex relationship between the esophagus and the LA posterior wall. This anatomic information is obtained from the MDCT, which provides submillimetric spatial resolution resulting in high precision. It offers 3D information and allows for accurate representation of the atrioesophageal contact surface, not only in the sagittal and coronal planes but also in the axial plane. Given that the image is projected in a 2-dimensional manner on the LA endocardial mesh, the isodistance map provides information regarding the proximity between both structures. The observed interindividual variability of the atrioesophageal relationship and of the esophageal trajectory within the mediastinum endorses the need to develop easy-to-implement strategies that enable use of this information during catheter ablation on the posterior LA wall, particularly as different energy delivery ablation protocols are put into practice.

Stability of esophageal position between procedures

High stability of the esophageal position was observed between procedures that were 6 (IQR 3 ± 9) months apart. No position shift was observed in 91% of patients, and only subtle changes were observed between the 2 interventions. This 3D observation not only confirms earlier reports on the stability of the esophagus within the mediastinum having a so-called "lying position"¹¹ but also the relative stability of the atrioesophageal relationship as analyzed by the esophageal fingerprint. This was further confirmed by the closest fingerprinted esophageal area (distance to LA posterior wall <1 mm), as no significant change was observed in the contact surface. In fact, the mediastinum being filled with multiple anatomic structures within the thoracic cage provides space for passage, but important mobility within it seems unlikely in the absence of deglutition or mechanical forces.

Stability of esophageal position during the same procedure

Conflicting evidence on the stability of esophageal position during a procedure has been reported. Piorkowski et al¹²

 Table 1
 Baseline characteristics of the study populations

	Group 1*: MDCT (n = 39)	Group 2: Multimodality (n = 100)
Age (y)	60 ± 10	61 ± 11
Male	26 (67)	64 (64)
Hypertension	23 (60)	39 (39)
Dyslipidemia	10 (26)	17 (17)
Diabetes	2 (5)	6 (6)
LVEF	59 ± 6	56 ± 7
LA diameter (mm)	44 ± 4	39 ± 6
BMI (kg/m^2)	29 ± 6	27 ± 4
CHADS-VASc score		
0	9 (23)	30 (30)
1	16 (41)	23 (23)
2	5 (13)	23 (23)
3	8 (20)	18 (18)
4	1 (3)	3 (3)
5	_	2 (2)
6	—	1 (1)
Time between MDCT	Median 6 (IQR	N/A
examinations (mo)	3–9)	
Ablation type		
First ablation	0	78 (78)
Redo ablation	39 (100)	22 (22)
AF type		
Paroxysmal	25 (64)	69 (69)
Persistent	12 (31)	26 (26)
Longstanding persistent	0 (0)	1 (1)
Atrial tachycardia/flutter	2 (5)	4 (̈́́́́́́́́́́́́́́́́́́́́

Values are given as mean \pm SD or n (%) unless otherwise indicated. BMI = body mass index; IQR = interquartile range; LA = left atrium; LVEF = left ventricular ejection fraction; MDCT = multidetector computed tomography; N/A = not applicable.

*For group 1, characteristics at redo.

showed the concordance of esophageal position between the preprocedural MDCT and the electroanatomic map. Sherzer et al¹³ found stability of esophageal position relative to the spine by means of an ablation catheter in the esophagus during the whole procedure in patients under general anesthesia. Another study found that esophageal position was relatively stable before and after ablation using 3D rotational angiography for the PVs and visualization of the esophagus via peroral administration of a contrast agent.¹⁴ However, whether esophageal movement is stimulated by the swallowing of barium and whether general anesthesia has any influence on esophageal motility with a postulated hypothesis that the esophagus has a resting position are unknown. Real-time observation of the esophageal position is feasible with intracardiac echocardiography, but this costly option is not widely available.

We have also come to understand that the esophagus is a wide structure (mean width 28.1 ± 4.1 mm). This explains why previous publications described a discordant position between esophageal FAM and MDCT-derived esophageal position as observed by means of a gastric tube placed on 2 consecutive occasions,¹⁵ as the positioning of a tube within

the esophageal width is random and can differ greatly in the coronal plane.

The present study found good stability of esophageal position between pre- and periprocedural multimodality imaging. In only 8 of 100 patients (8%) was a discordance between at least 2 of the imaging techniques observed. One patient was not sufficiently sedated and coughed on the tube during the procedure. Another patient had a superior vena cava circumferential ablation that motivated local stimulation to check for phrenic nerve anatomy, which presumably could have modified the esophageal position through diaphragmatic contraction (Figure 5). In 1 patient, the transesophageal probe was inadvertently left in place during the ablation procedure. Finally, one patient had an electrical cardioversion during the procedure. Overall, 16 patients (16%) were cardioverted, and in only 1 case did the position differ. There was no apparent cause for discordance in the 5 remaining cases.

Clinical implications

This study describes MDCT-derived 3D isodistance maps to better understanding of the atrioesophageal allow relationship, which is fundamental for improving procedural safety. Our results emphasize the importance of multimodality imaging to integrate the complexity of the anatomic relationships and allow safer energy delivery without compromising the efficacy of ablation lesions in the LA posterior wall. The esophageal fingerprinted isodistance map image is a novel tool that could be used to modulate energy delivery and modify the course of RF ablation lines, cryoballoon placement, and laser application with the aim of improving safety. However, this application is merely hypothetical, and further prospective studies are underway to prove this utility (AWESOME-AF randomized trial; ClinicalTrials.gov Identifier: NCT04394923).

Study limitations

The main limitation of this study is the lack of a control group for comparison (eg, patients under conscious sedation). The study was observational and was not designed to allow for sufficient power to find predictors of significant esophageal position modification between 2 procedures or during the same procedure. In the multimodality group, a limitation is the intrinsic discordances between these techniques. The most accurate is CT because it delineates the actual anatomy as acquired (mean esophageal width 27.2 \pm 4.3 mm). The other techniques are approximative, and all have limitations. The esophageal image rendered by UNIVU with the TEE probe in place is unavoidably thinner because it relies on the probe width (15 mm). For the esophageal FAM, a limitation is that esophageal anatomy can be deformed if significant force is applied to the catheter. Thus, it would have been more accurate to compare esophageal position before, during, and after the procedure using the same technique (eg, esophageal FAM), yet this was not plausible in the clinical



Figure 5 Group 2 (multimodality) results. **Top:** High image correlation. **Bottom:** Low image correlation). **1:** Anteroposterior UNIVU acquisition with transesophageal echocardiographic probe in place. **2:** Correlation between MDCT and CARTOUNIVU. **3:** Correlation between MDCT and esophageal FAM. **4:** Correlation between MDCT and esophageal FAM. FAM = fast anatomic mapping; MDCT = multidetector computerized tomography.

setting of the present study. Nevertheless, the presented results challenge the common belief that the esophagus undergoes significant movement. As a matter of fact, such movements are infrequent and subtle. Furthermore, these results might not be extrapolated to patients undergoing ablation under conscious sedation (as opposed to general anesthesia) or with more time-consuming ablation protocols, as the median procedural time was under 1 hour. Moreover, MDCT data of the index procedure were not available for redo patients in the multimodality group. Ultimately, the question of esophageal position needs to be evaluated by a constant intraoperative monitoring technique such as intracardiac echocardiography (ICE). However, the technique is not widespread, and data regarding real-time esophageal position with ICE are not yet available. Research is underway to test the clinical utility of the esophageal fingerprinted isodistance map in the modulation of RF delivery and design of PV ablation lines (AWESOME-AF; ClinicalTrials.gov Identifier: NCT04394923).

Conclusion

There is high stability of esophageal position between procedures and from the beginning to the end of an AF ablation procedure. The 3D fingerprinted isodistance atrioesophageal map can be obtained from MDCT scan and integrated into the navigation system. It allows for direct periprocedural estimation of the distance between the esophagus and the LA posterior wall. **Funding Sources**: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Patient Consent: All participants included in the study provided written informed consent.

Ethics Statement: The study complied with the Declaration of Helsinki, and the local ethics committee approved the study protocol.

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