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1 TITLE:

2 Three-Dimensional Echocardiographic Method for the Visualization and Assessment of

3 Specific Parameters of the Pulmonary Veins

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KEYWORDS:

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SUMMARY:

The dimensions of the pulmonary veins (PV) are important parameters when planning pulmonary vein isolation. 2D transoesophageal echocardiography can only provide limited data about the PVs; however, 3D echocardiography can evaluate relevant diameters and areas of the PVs, as well as their spatial relationship to surrounding structures.

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ABSTRACT:

The dimensions of the pulmonary veins are important parameters when planning pulmonary vein isolation (PVI), especially with the cryoballoon ablation technique. Acknowledging the dimensions and anatomical variations of the pulmonary veins (PVs) may improve the outcome of the intervention. Conventional 2D transoesophageal echocardiography can only provide limited data about the dimensions of the PVs; however, 3D echocardiography can further evaluate relevant diameters and areas of the PVs, as well as their spatial relationship to surrounding structures. In previous literature data, parameters influencing the success rate of PVI have already been identified. These are the left lateral ridge, the intervenous ridge, the ostial area of the PVs and the ovality index of the ostium. Proper imaging of the PVs by 3D echocardiography is a technically challenging method. One crucial step is the collection of images. Three individual transducer positions are necessary to visualize the important structures; these are the left lateral ridge, the ostium of the PVs and the intervenous ridge of the left and right PVs. Next, 3D images are acquired and saved as digital loops. These datasets are cropped, which result in the en face views displaying spatial relationships. This step can also be employed to determine the anatomical variations of the PVs. Finally, multiplanar reconstructions are created to measure each individual parameter of the PVs.

Optimal quality and orientation of the acquired images are paramount for the appropriate assessment of PV anatomy. In the present work, we examined the 3D visibility of the PVs and the suitability of the above method in 80 patients. The aim was to provide a detailed outline of the essential steps and potential pitfalls of PV visualization and assessment with 3D echocardiography.

INTRODUCTION:

The drainage pattern of the pulmonary veins (PV) is highly variable with 56.5% variation in the average population¹. Evaluation of the PV drainage pattern is crucial when planning PV isolation (PVI), which is the most common interventional treatment of atrial fibrillation nowadays²⁻⁴. Although radiofrequency catheter ablation has been the standard technology for achieving PVI, the cryoballoon (CB)-based ablation technology (CA) is an alternative method requiring less procedural time. The technique is less complicated compared with radiofrequency ablation^{5,6}, while the efficacy and safety of CA are similar to those of radiofrequency ablation⁷.

The rate of procedural PV occlusion by the CB and the continuous circumferential extension of tissue injury in the PV ostium determines the permanent success of PVI after CA. One of the main determinants of PV occlusion is the variation of PV anatomy. In recent, computed tomography- (CT) and cardiac MRI-based studies, several PV parameters were identified with predictive values of short and long term success rates following CA. These parameters included variations of both the PV anatomy (left common PV, supernumerary PVs⁸⁻¹⁰, ostial area, ovality index^{8,11-13}) and its surroundings (intervenous ridge^{8,14-16}, thickness of left lateral ridge^{8,9,17}).

Although conventional 2D echocardiography is not suitable for displaying and measuring most of the above parameters, three-dimensional transesophageal echocardiography (3D TEE) seems to be an alternative tool to visualize the PVs, as demonstrated in previous literature data^{18,19}.

Furthermore, 3D TEE prior to PVI brings additional value compared to CT or MRI, as it not only provides data on PV characteristics for procedural design, but also clarifies whether a thrombus in the left atrial appendage (LAA) is present. This investigation is especially important prior to PVI. At the same time, 3D TEE requires less time, its procedural cost is low, and it does not expose the patient and the medical staff to radiation.

In the past, several types of CBs existed with different sizes, which made it difficult to extrapolate how the various parameters of the PVs influence the success rate of CA. Today, the newly introduced second-generation CB is used for CA, which only exists in one size. Thanks to its improved cooling effect, the second-generation CB offers a much higher performance compared to the first-generation CB²⁰, which further highlights the importance of PV anatomy and interventional planning before PVI.

PROTOCOL:

 All the patients signed informed consent before examination according to approval of the local ethical committee (OGYÉI/12743/2018).

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1. Preparation

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1.1. Start the examination with patient preparation: ensuring at least a 4-hour fasting status, questionnaire about problems with swallowing and known upper gastrointestinal diseases.

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104 1.2. Ensure that written informed consent is read and signed.

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106 1.3. Prepare an intravenous line before the examination.

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108 1.4. Position the patient in a left lateral decubitus position.

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110 1.5. Administer mild sedation using intravenous midazolam (2.5-5 mg).

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112 1.6. Monitor ECG and oxygen saturation.

2.1. Visualization of the left PVs

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114 **2.** Image acquisition

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2.1.1. Insert the probe into the oesophagus at approximately 30-40 cm from the front teeth.

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2.1.2. In the upper (or mid) transoesophageal probe position visualize the LAA using 2D image acquisition at 20-45°.

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124 2.1.3. Turn the probe slightly clockwise and change the crystal angulation to 60-80° in order
 125 to centralize the LAA on the image.

126

127 2.1.4. Click the full volume button in order to applying full volume 3D acquisition.

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2.1.5. Adjust the image's lateral and elevational width to display the LAA and the left upper PV. This enhances visualization of the left lateral ridge.

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132 2.1.6. Optimize the image quality (adjusting the depth and gain, applying harmonic imaging).

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2.1.7. Record a one-beat (if feasible, multibeat) loop with 2 cardiac cycles.

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2.1.8. Change the angulation to approximately 120° on the 2D image to centralize the LAA.

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2.1.9. Turn the probe slightly counterclockwise and apply anteflexion to visualize the ostia of the left PVs.

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2.1.10. Apply color Doppler-coded imaging to confirm that both the upper and lower PVs are
 visible.

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2.1.11. Click the full volume button in order to applying full volume 3D acquisition.

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2.1.12. Adjust the image's lateral and elevational width to display the left PVs. This enhances
 visualization of the ostia of the left upper and lower PVs and the intervenous ridge.

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2.1.13. Control dataset quality. Check the recorded dataset. If the dataset does not contain both the upper and lower PVs, change the patient position by further tilting to the lateral position, and repeat the procedure from step 2.1.8.

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2.1.14. Acquire 3D full volume datasets from the left PVs: one-beat (if feasible, multibeat)
 loop with 2 cardiac cycles.

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2.1.15. Confirm the visibility of PV ostia by cropping the image to the upper or lower PV ostium, respectively. The lower PV ostium requires a specifically careful confirmation. It is not unsual, that some parts of the ostium are outside the 3D dataset due to anatomical reasons, e.g. angulation or close proximity to the transducer.

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2.1.16. In case the image is not suitable for visualizing the complete PV structure, repeat the procedure from step 2.1.10. Change the lateral or elevational width of the 3D dataset, if necessary.

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2.2. Visualization of the right PVs

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2.2.1. Switch back to 2D mode and focus the image to the LAA at 45° upper (or mid) oesophageal probe position.

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171 2.2.2. Turn the probe clockwise and move the probe head to anteflexion position in order
 172 to visualize the right PVs.

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2.2.3. Apply color Doppler-coded imaging to confirm that both the upper and lower PVs are
 visible.

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2.2.4. Click the full volume button in order to applying full volume 3D acquisition.

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2.2.5. Adjust the image's lateral and elevational width to display the right PVs. This enhances visualization of the ostia of the right upper and lower PVs and the intervenous ridge. This image can be used to identify the presence of supernumerary PVs.

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2.2.6. Acquire 3D full volume datasets from the right PVs: one-beat (if feasible, multibeat)
 loop with 2 cardiac cycles.

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2.2.7. Confirm the visibility of PV ostia by cropping the image to the upper or lower PV ostium, respectively. The lower PV ostium requires a specifically careful confirmation. It is not unusual that some parts of the ostium are outside the 3D dataset due to anatomical

reasons (e.g., angulation or close proximity to the transducer).

2.2.8. If the dataset does not contain both the upper and lower PVs, patient position should be changed by further tilting to the right position, and the procedure should be repeated from step 2.2.1. Change the lateral or elevational width of the 3D dataset, if necessary.

3. 3D image reconstruction and measurements

197 3.1. Offline 3D multiplanar reconstruction

3.1.1. Activate the 3DQ software in QApps panel.

3.1.2. Select a frame in diastolic phase for the measurements. For standardization, it is recommended to select a frame timed to the T wave.

3.1.3. Set the two perpendicular planes to the requested structure (left lateral ridge or each PVs ostium) and adjust plane direction while the 3rd plane represents the *en face* view of the examined structure.

3.1.4. On the left panel, select the measurement option. The *en face* view is suitable for measurements (diameter, area, distance).

REPRESENTATIVE RESULTS:

Using the above-described image acquisition protocol, the first step is to visualize the left atrial appendage (LAA) using 2D acquisition (**Figure 1**). The probe is in the upper (or mid) transoesophageal position at 20-45°. The image shows the LAA. The left lateral ridge and the left upper PV is displayed at 60-80° (**Figure 2**), and then the 3D dataset is acquired and confirmed by cropping the dataset in order to visualize the LAA and the left lateral ridge with the left upper PV ostium (**Figure 3**). If the dataset does not encompass the whole structure of the LAA and the left lateral ridge, the image acquisition is repeated while changing the probe angulation, flexion or changing the patient position.

The next step is to visualize the left PVs. The probe angulation is changed to at around 120° to centralize the image to the LAA, and then the probe is turned slightly counterclockwise while moving the probe head to anteflexion. When the left PV ostium is visible (**Figure 4**), color Doppler is used to confirm that both the upper and lower PV is visible (**Figure 5**). Then the 3D dataset is acquired and confirmed by cropping the image to left upper and lower PV ostia with the intervenous ridge (**Figure 6**). If the dataset does not encompass the whole structure of the left PV ostium, image acquisition should be repeated while changing the probe angulation, flexion or changing the patient position.

The next step is the visualization of the right PVs. The probe angulation is changed to approximately 45° to centralize the image to the LAA, and then the probe is turned slightly clockwise while moving the probe head to anteflexion. When the right PV ostium is visible (Figure 7), color Doppler-coded imaging is used to confirm that both the upper and lower PV is clearly visible (Figure 8). Then the 3D dataset is acquired and confirmed by cropping the image to the right upper and lower PV ostia with the intervenous ridge (Figure 9 and Figure

). If the dataset does not encompass the whole structure of the right PVs ostia, image acquisition should be repeated while changing the probe angulation, flexion or changing the patient position.

The next step is to prepare the 3D dataset offline and perform the measurements. The selected 3D dataset is opened in a dedicated platform-specific or a vendor-independent software for multiplanar reconstruction of the 3D images. First, one should select a frame timed to the T wave, and then two perpendicular planes are positioned to the PV ostia. The 3rd plane represents the *en face* view of the ostium (**Figure 11**), which is suitable to measure dimensions (distances, area). If the two perpendicular planes are fitted to the ridge, the widths of the ridges can be measured.

FIGURE AND TABLE LEGENDS:

Figure 1. 2D view of the left atrial appendage at 22°. Left atrial appendage

Figure 2. 2D view of the left atrial appendage at 75°. (A) Left atrial appendage; (B) Left lateral ridge; (C) Left upper pulmonary vein

Figure 3. 3D reconstruction of the left lateral ridge and the left upper pulmonary vein. (A)
Ostium of the left upper pulmonary vein; (B) Left lateral ridge; (C) Left atrial appendage

Figure 4. 2D view of the left pulmonary veins at 122°. (A) Left lower pulmonary vein; (B)
Intervenous ridge; (C) Left upper pulmonary vein

Figure 5. 2D color-coded image of the left pulmonary veins at 122° to confirm pulmonary venous flow. (A) Left lower pulmonary vein; (B) Left upper pulmonary vein

Figure 6. 3D reconstruction of the left pulmonary veins. (A) Ostium of the left lower pulmonary vein; (B) Intervenous ridge; (C) Left upper pulmonary vein; (D) Left lateral ridge; (E) Left atrial appendage

Figure 7. 2D view of the right pulmonary veins at 45°. (A) Right lower pulmonary vein; **(B)** Intervenous ridge; **(C)** Right upper pulmonary vein

Figure 8. 2D with color-coded image of the right pulmonary veins at 45° to confirm pulmonary venous flow. (A) Right lower pulmonary vein; (B) Intervenous ridge; (C) Right upper pulmonary vein

Figure 9. 3D reconstruction of the right pulmonary veins focusing on the right upper vein.

(A) Right upper pulmonary vein; (B) Intervenous ridge; (C) Right intermediate pulmonary vein (example for a supernumerous drainage pattern on the right side)

Figure 10. 3D reconstruction image of right pulmonary veins tilting the focus toward the right lower PV. (A) Right upper pulmonary vein; (B) Intervenous ridge; (C) Right intermediate pulmonary vein (example for supernumerous drainage pattern in the right side); (D) Right lower pulmonary vein **Figure 11.** Multiplanar reconstructed 3D images of the left upper pulmonary venous ostium. (A,B) Two perpendicular planes show the left upper PV longitudinally. The dotted lines represent the cutting planes. The blue one was fitted to the PV's ostium. (C) Short axis view shows the en face view of the left upper pulmonary vein; (D) 3D dataset with a cutting plane.

Figure 12. Multiplanar reconstructed 3D images of the left lateral ridge and left upper pulmonary vein. (A) Left atrial appendage (longitudinal view – panel A; cross-sectional view – panel C); (B) Left lateral ridge (longitudinal view – panel A; cross-sectional view – panel C); (A) Left upper pulmonary vein (longitudinal view – panel A; cross-sectional view – Panel C)

DISCUSSION:

Here, we demonstrate a step-by-step methodology to study the PVs, their surrounding structures and anatomical characteristics with 3D echocardiography. The above described method for 3D imaging of the PVs is an easily standardizable method, which provides high quality 3D images in most patients suitable for precise measurements. Optimal quality and orientation of the acquired images are paramount for the appropriate assessment of PV anatomy. The 3D reconstructed images enhance the visualization of the PV drainage pattern and its anatomical variability, which may influence the success rate of PVI with CA.

3D imaging of the PVs overcomes the technical limitations of conventional 2D transoesophageal echocardiography and makes the 3D transoesophageal echocardiography method allow to substitute cardiac MRI or CT imaging of PVs before PVI, especially if the last imaging techniques are not available.

The important step is changing the patient position during the examination if the visibility of the PVs is not satisfactory. This modification contributes to improve the visibility of the PVs. Displaying the right lower PV is the most challenging part of this method. If some parts of the PV's ostium are outside the 3D dataset due to anatomical reasons (e.g., angulation or close proximity to the transducer), the precise measurement of PV's parameter will not be possible, which is the limitation of this method.

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DISCLOSURES:

The authors report no conflicts of interest.

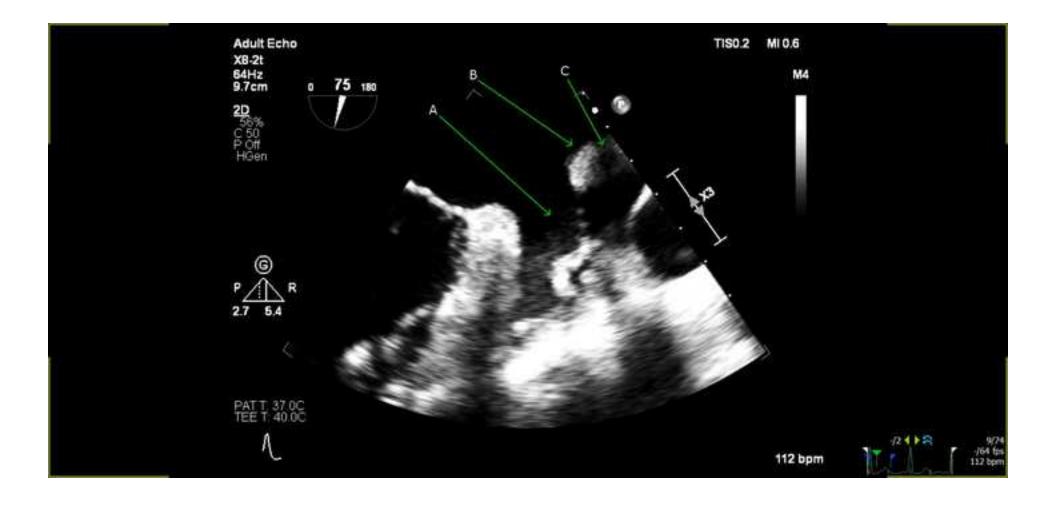
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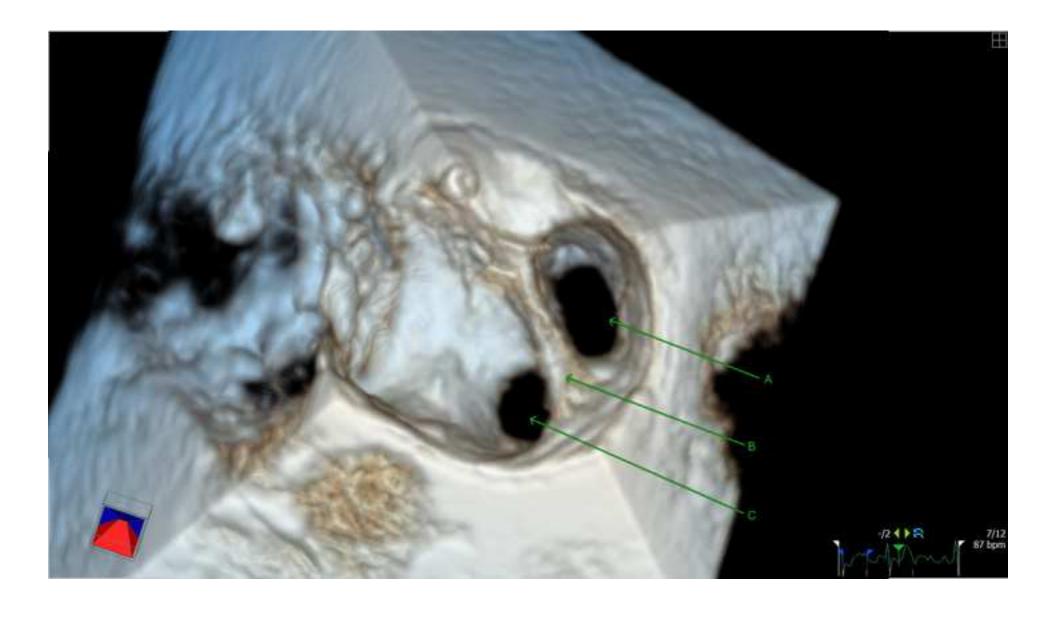
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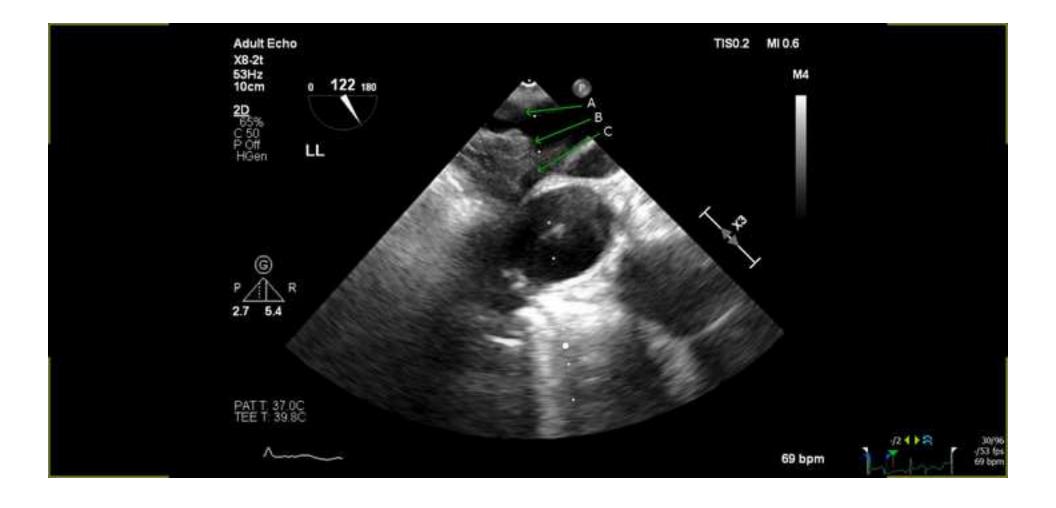
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- Association (EHRA), a registered branch of the European Society of Cardiology (ESC) and the
- 338 European Cardiac Arrhythmia Society (ECAS); and in collaboration with the American College
- of Cardiology (ACC), American Heart Association (AHA), the Asia Pacific Heart Rhythm
- 340 Society (APHRS), and the Society of Thoracic Surgeons (STS). Endorsed by the governing
- bodies of the American College of Cardiology Foundation, the American Heart Association,
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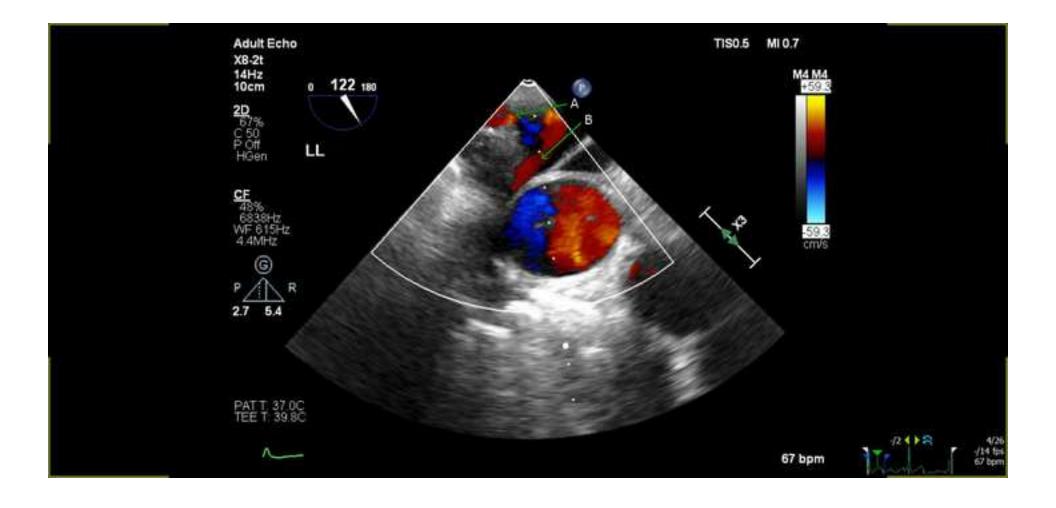
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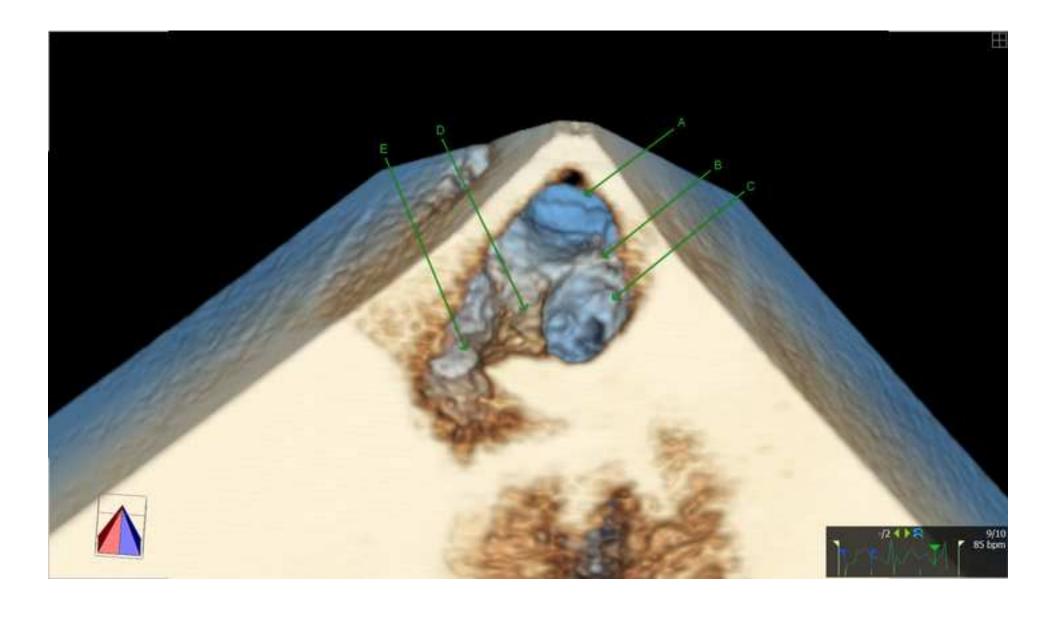


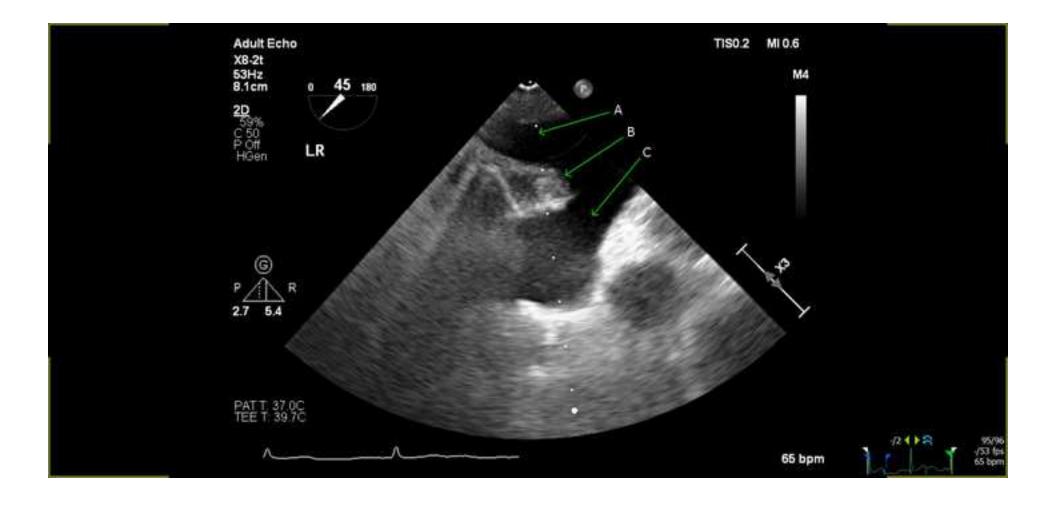


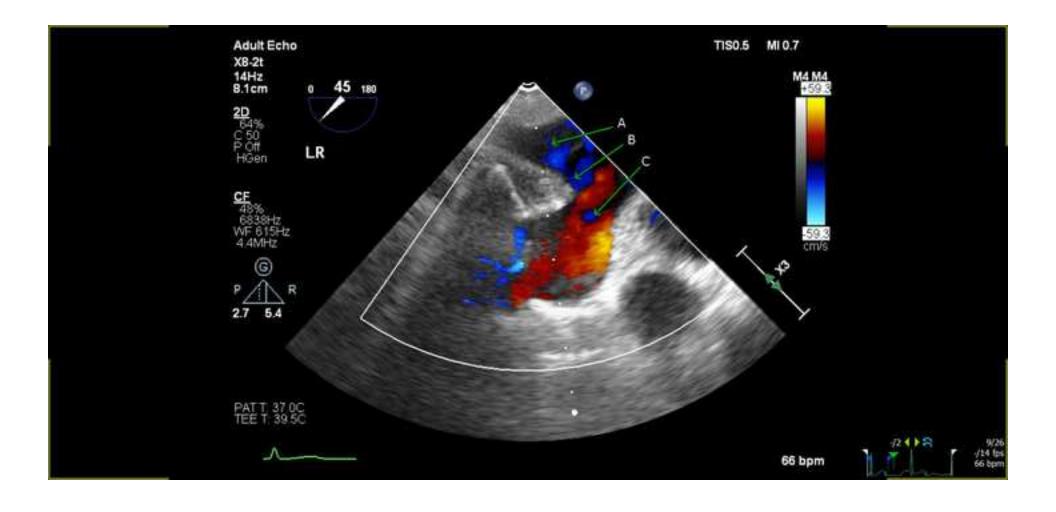


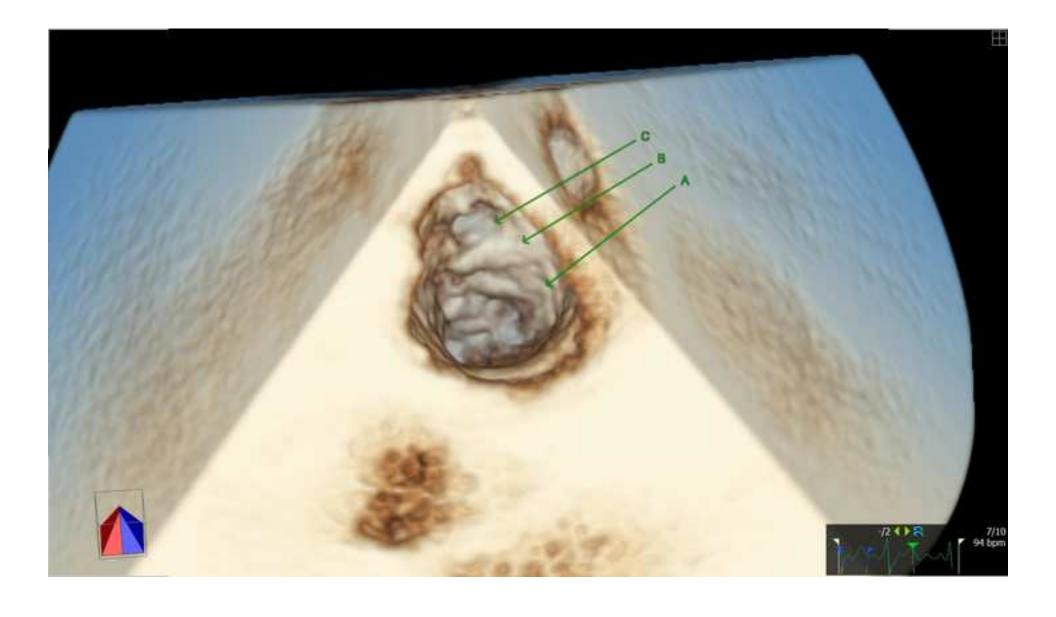


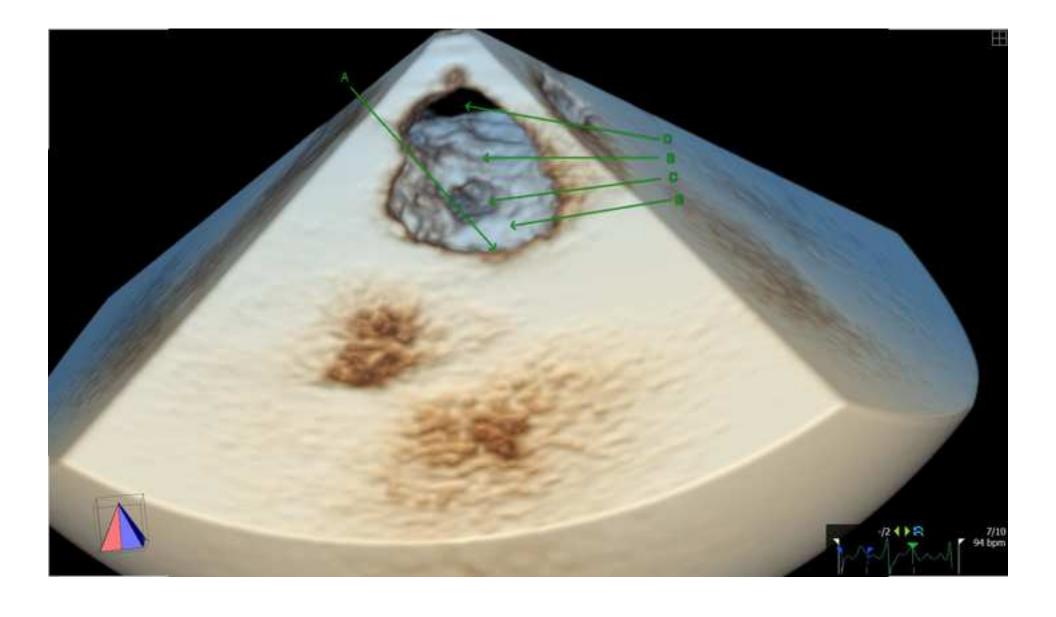


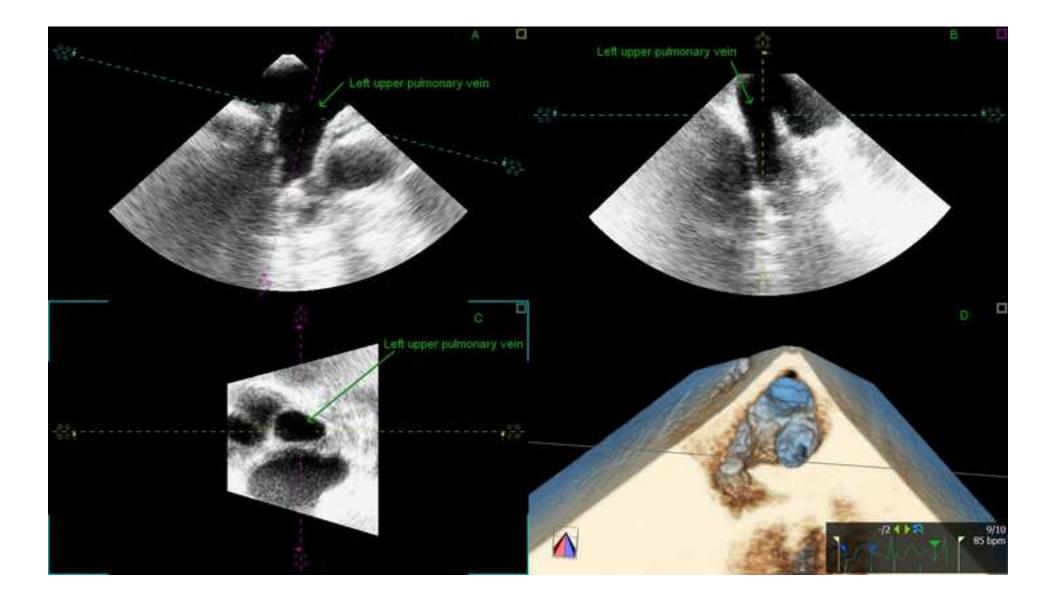


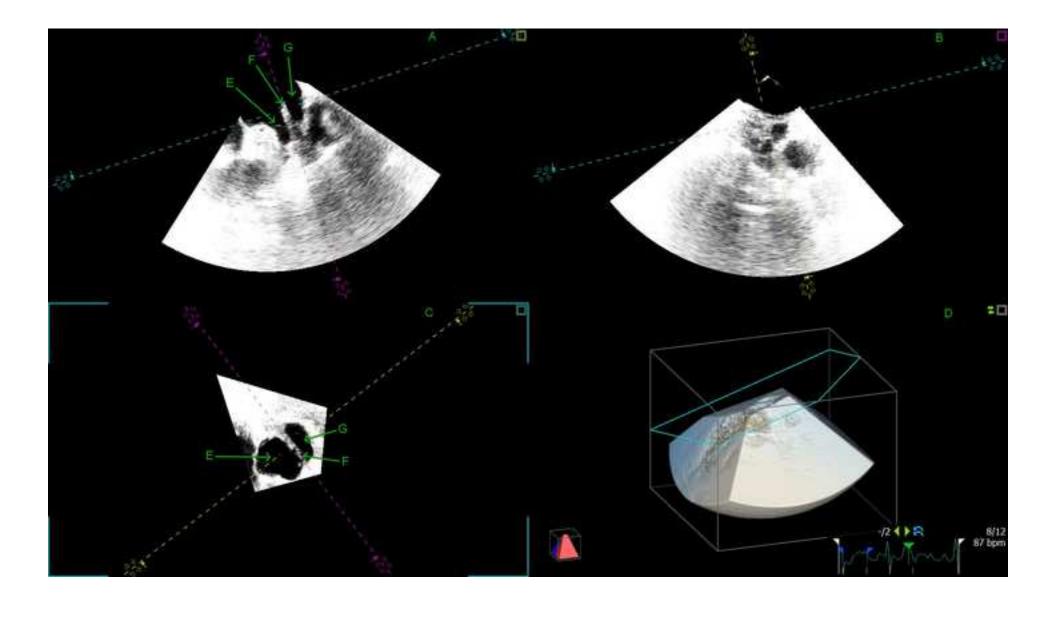












Name of Material/Equipment	Company	Catalog Number	Comments/Description
4D Cardio-view 3 software	Tomtec Imaging Systems GmbH		
Epiq 7G scanner	Philips		
Q-Lab Software	Philips		
X5-1 transducer	Philips		

Responses

Editorial Comments:

- Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.
- Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the guidelines of your institutions human research ethics committee.

The ethics statement and the number of permission were provided in the text.

Protocol Language:

- 1) Please use complete sentences throughout.
- 2) The JoVE protocol should be almost entirely composed of numbered short steps (2-3 related actions each) written in the imperative voice/tense (as if you are telling someone how to do the technique, i.e. "Do this", "Measure that" etc.). Any text that cannot be written in the imperative tense may be added as a brief "Note" at the end of the step (please limit notes). Please re-write your ENTIRE protocol section accordingly. For example: "Probe insertion in the oesophagus at approximately 30-40 cm from the front teeth." Should be "Insert the probe into the oesophagus approximately 30-40 cm from the front teeth."

All the suggested modifications were re-written.

• Protocol Detail: Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

I added more specific details in some protocol steps where it was applicable.

• Protocol Numbering: There must a one-line space between each protocol step.

Corrected.

- Protocol Highlight: Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.
- 1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
- 2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

- 3) Please highlight complete sentences (not parts of sentences). Include subheadings and spaces when calculating the final highlighted length.
- 4) Notes cannot be filmed and should be excluded from highlighting.

I highlighted the essential steps of the protocol which will suitable for the filming.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

The discussion section was re-written according to the above mentioned details.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

All the used figures are original figures which have not used previously.

Comments from Peer-Reviewers:

Reviewer #1:

Manuscript Summary:

The authors described a step-wise protocol using the TEE to obtain 3-D anatomic information of the LA, LAA and the pulmonary veins.

Major Concerns:

none.

Minor Concerns:

- 1. As you have mentioned the importance of the PV anatomy during the PVI/ablation procedure, perhaps you may have or provide some TEE images during the cryoballoon-based PVI? e.g. TEE showing each PV occluded by the cryoballoon.
- 2. You may added one figure showing the CT or MRI Reconstruction of the LA, LAA and the PVs, allowing the readers more readily to understand the 3-D Echocardiogram.
 - 1. We use the 3D TEE to visualize the PVs anatomy before the PVI which helps the planning the procedure. Our PVI procedural protocol does not include TEE guidance, so we do not have 3D TEE images showing the PVI steps.

2. 3D TEE easier to access than cardiac MRI or CT, so we use the 3D TEE substituting the cMRI or CT. That is why I could provide TEE images only to visualize the PVs.

Reviewer #2:

Manuscript Summary:

This manuscript describes visualizing PV using 3D echo in order to guide PVI for atrial fibrillation. The description is thorough and easy to follow.

Minor Concerns:

While the described technique is not novel, the detailed approach and the troubleshooting tips are helpful

It is not the usual way to visualize the PVs by 3D TEE to characterize the PV's anatomical aspects for the planning the procedure. We tried some modifications in the method which led to improve the visualization of the PVs.