Guidelines

Guideline on the Clinical Use of Ultrasound-Enhancing Agents in Cardiology Applications – 2026

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Note: These guidelines are for information purposes and should not replace the clinical judgment of a physician, who must ultimately determine the appropriate treatment for each patient.

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1. Introduction

In the last two decades, new perspectives have opened for a better evaluation of cardiovascular pathophysiology, especially coronary macro- and microvascular pathophysiology, due to the development of ultrasound-enhancing agents (UEAs) based on encapsulated microbubbles prepared for intravascular injections.

These microbubbles behave similarly to red blood cells, remain exclusively intravascular, and freely circulate through the macro- and microcirculation, causing opacification of cardiac chambers and the capillary microcirculation. The primary mechanism whereby these microbubbles produce blood-pool enhancement is the introduction of multiple gas-liquid interfaces into the circulation, increasing ultrasound reflection by creating small areas with significant differences in impedance. Modern microbubbles used for diagnostic purposes are approximately 2μm in diameter (smaller than half the width of a human capillary) and resonate at frequencies used in diagnostic ultrasound for humans, around 2 MHz.[1-4]

Microbubbles were first described by Gramiak and Shah in 1969, when they accidentally injected agitated indocyanine green solution (Cardiogreen®) into the ascending aorta of a patient during cardiac catheterization and detected intense clouds of echoes. Subsequent studies showed these echoes resulted from air bubbles in the injected solution, generated by agitation, leading to cavitation.[5]

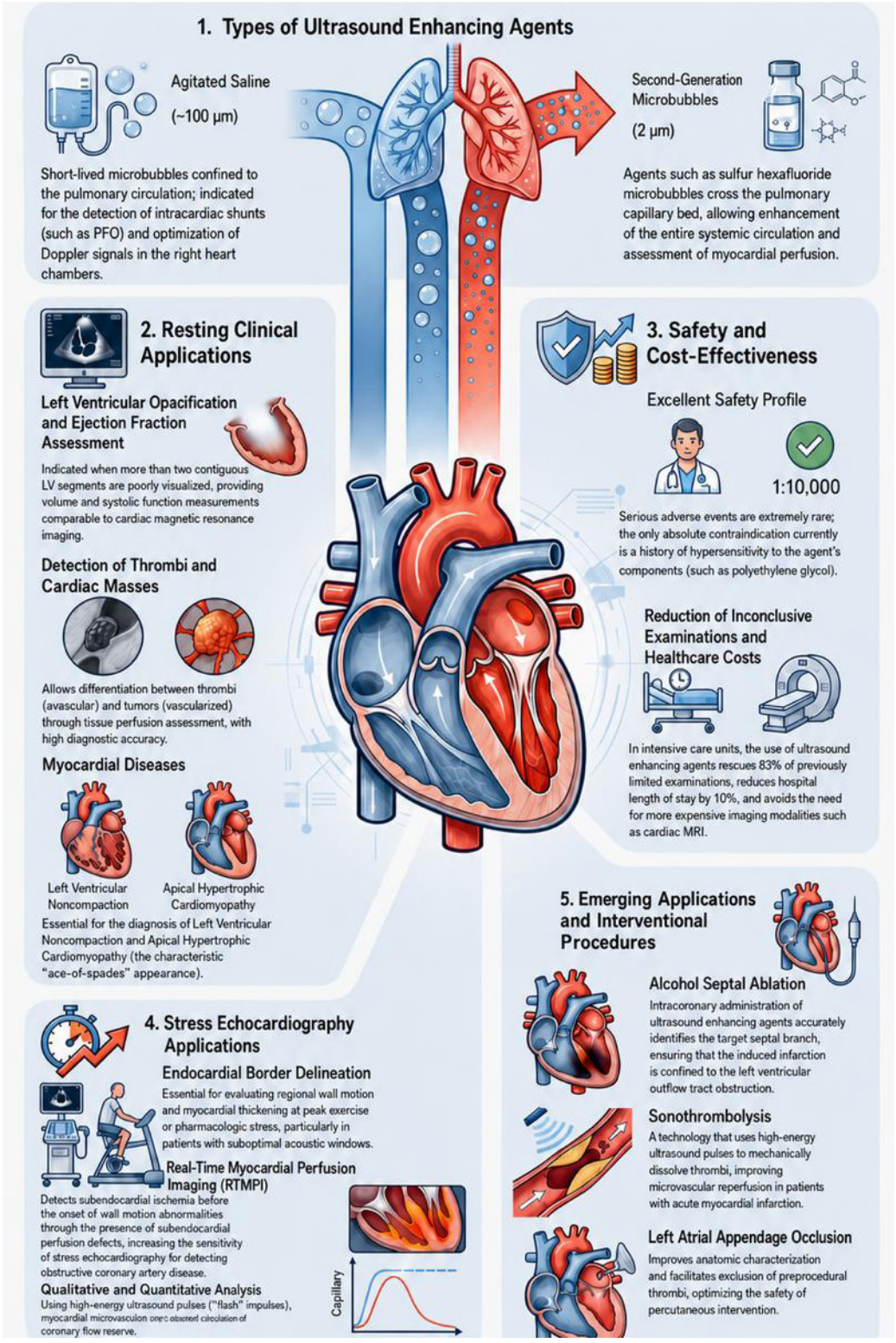
This description resulted in the first known enhancing agents restricted to the pulmonary circulation, formed by microbubbles of a solution containing a mixture of agitated 0.9% saline solution, a small amount of the patient's own blood (optional), and ambient air (mainly nitrogen and oxygen). These microbubbles do not cross the pulmonary capillary barrier and, when injected via the peripheral intravenous route, opacify only the right side of the heart, unless intracardiac or intrapulmonary shunting is present.

The development of sonication – a process that exposes the enhancing solution to high ultrasonic energy, around 20 000 Hz – by Steve Feinstein in 1984 made it possible to generate air-filled microbubbles smaller than a capillary, capable of opacifying the left heart by crossing the pulmonary capillary bed; these microbubbles are defined in the present document as "whole-circulation UEAs". Feinstein's studies led to development of the first-generation UEA known as Albunex® (Mallinckrodt Medical Inc., St. Louis, USA), produced by sonication of a solution containing serum albumin, glucose and ambient air,[6] capable of transpulmonary passage and visualization of the systemic vasculature after intravenous injection. Other first-generation UEAs were also marketed in several countries and, with few exceptions, are no longer available.

Subsequently, second-generation UEAs containing gases with higher molecular weight and lower diffusivity, such as perfluorocarbons, were developed, resulting in more stable microbubbles capable of longer persistence in the pulmonary and systemic circulation and permitting clinically useful ultrasound image enhancement.[7] Examples of these second-generation

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Central Illustration: Guideline on the Clinical Use of Ultrasound-Enhancing Agents in Cardiology Applications – 2026



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UEAs currently marketed in several countries include Optison®, Definity® (or Luminity®)*, SonoVue® (or Lumason®)* and Sonozoid®. Note: The same second-generation UEAs may have different trade names depending on the country where they are marketed.

Currently, the main clinical indications for microbubble echocardiography are:[1]

- Microbubbles obtained from agitated saline solution (~100 μm in diameter), here defined as "microbubbles for exclusive pulmonary circulation enhancement": indicated to improve visualization of the pulmonary circulation, in the investigation of intracardiac shunting (primarily patent foramen ovale and interatrial communications of other etiologies), and optimization of Doppler signal in the right heart cavities. They are prepared by vigorously transferring the contents of a 10-mL syringe to another in a closed system, using a mixture containing 9.0 mL of 0.9% saline solution, 0.7 mL of the patient's own blood (optional), and 0.3 mL of ambient air. This results in an "agitated saline solution" containing a heterogeneous mixture of short-lived microbubbles of approximately 100 μm average diameter, which do not cross the pulmonary capillary bed, which has a mean diameter of 10 μm.
- Microbubbles ~2 μm in diameter, here defined as "microbubbles for whole-circulation enhancement": indicated for opacification of the whole circulation, in cardiology for left ventricular opacification and improved endocardial border delineation, permitting quantitative evaluation of ejection fraction, detection of intracavitary thrombi and optimization of Doppler signal, including for measurement of pulmonary artery pressures; additionally, microbubbles detected within the myocardium allow non-invasive assessment of myocardial ischemia and detection of perfusion defects in acute and chronic coronary syndromes. These UEAs are commercially available.

2. Ultrasound-Enhancing Agents Available on the Global Market

2.1. The Use of Ultrasound-Enhancing Agents for Exclusive Pulmonary Circulation Enhancement (100-μm Microbubbles Based on Agitated 0.9% Saline Solution)

2.1.1. Assessment of Intracavitary Shunts

The assessment of intracardiac shunts has significant clinical relevance, since ischemic stroke is one of the leading causes of morbidity and mortality in the Brazilian population and worldwide, affecting about one-third of the economically active population and accounting for approximately 10% of all deaths in Brazil.[8] Despite extensive investigation, in around 30% to 40% of cases its etiology is not clarified, and it is then called cryptogenic stroke.[9] This situation is usually even higher in the young population, making it imperative to investigate paradoxical embolism, the main predisposing factor for which is patent foramen ovale (PFO), present in approximately 25% of the population.[9]

Despite the superiority of transesophageal echocardiography (TEE) in detecting shunts and in anatomical evaluation of the interatrial septum, transthoracic echocardiography (TTE) plays a relevant role in screening these patients and in quantifying transseptal atrial flow. Xu et al. reported that TTE with agitated 0.9% saline solution has a sensitivity of 89.4% and a specificity of 75% for detecting PFO, while TEE with agitated 0.9% saline solution achieves a sensitivity of 98.4% and a specificity of 100%.[10]

Although color flow mapping can be used to detect atrial septal defects, this technique is not always sufficient, especially in cases of widely mobile aneurysms, where septal movement itself can cause artifacts that lead to false positives. UEAs exclusively for the pulmonary circulation provide enhancement without traversing the pulmonary capillaries, and are thus useful for evaluating and quantifying paradoxical right-to-left shunts, detecting residual shunts after occlusion of the atrial septal defect, and even detecting arteriovenous shunts of the pulmonary parenchyma, which, unlike the former, are characterized by microbubbles becoming visible in the left cardiac chambers only 4 or more heartbeats after they reach the right atrium.

The mixture should be injected into an upper limb vein, preferably on the right side when the patient is lying in the left lateral position for image acquisition. In addition, optimal visualization of the interatrial septum through a shortened apical or subxiphoid window and the use of second harmonic imaging to optimize bubble detection are important considerations for better evaluation.[11] The test is considered positive for intracardiac shunt when microbubbles are present within the left atrium before the 4th heartbeat following their appearance in the right atrium. Ideally, the microbubbles will be visualized crossing the interatrial septum through the PFO,[12] a finding that is most easily made on TEE. If no microbubbles are detected in the left cardiac chambers, provocative maneuvers are recommended to increase right atrial pressure, since the communication may close functionally under normal left atrial pressure.[13] The Valsalva maneuver is the most common and effective provocative technique, but coughing or an abdominal thrust can also be used.[14,15] Additionally, the agitated saline solution can be injected into a peripheral IV line in the lower limbs, allowing the UEA to progress through the inferior vena cava and reach the right atrium with flow directed to the interatrial septum. This strategy is particularly useful in the presence of a prominent Eustachian valve. The appearance of microbubbles in the left atrium after a delay of four or more cardiac cycles suggests the presence of an intrapulmonary shunt, such as pulmonary arteriovenous malformations, which can often be confirmed by TEE through visualization of the microbubbles entering the left atrium via the pulmonary veins. The number of heartbeats used as a cutoff point for detection of cardiac or intrapulmonary shunts should not be considered dogma. The associated clinical and echocardiographic features should be evaluated on a case-by-case basis, since agitated-saline UEAs may appear earlier with pulmonary arteriovenous shunts in high-output states and later in cases of low cardiac output, or, through a PFO, detected by coughing or Valsalva maneuvers performed a few heartbeats after their arrival in the right heart.[14] In a subjective classification, taking into account the presence of these microbubbles in the left atrium or ventricle in a single image frame, a shunt is considered slight (grade 1) when there are 1 to 9 bubbles; moderate (grade 2) when there are 10 to 30 bubbles; and severe (grade 3) when there are more than 30 bubbles

(Figure 1). The use of the respirometer, already attached to the electrocardiograph cable in most echocardigraphic machines, could assist in the temporal localization of these events.

2.1.2. Improvement of Doppler signal

According to the American Society of Echocardiography recommendations, intravenous injection of UEAs is also indicated to improve right-sided spectral Doppler recordings in patients with suboptimal studies, defined as when it is not possible to obtain adequate recordings. It is important to state that Doppler flow velocities do not change with the use of UEAs – it is only the Doppler signal that is enhanced.

Continuous wave Doppler is an essential part of the echocardiographic evaluation, allowing for the estimation of intracavitary pressures and transvalvular gradients. Agitated saline microbubbles enhance the signal from the blood, making them useful for improving the Doppler signal, particularly when tricuspid and pulmonary regurgitation signals are suboptimal or absent, thus enabling the estimation of systolic, mean, and end-diastolic pulmonary artery pressures.[16]

In a prospective study with 90 patients who underwent invasive pulmonary artery pressure measurement and simultaneous evaluation by echocardiography, the correlation between the invasive pulmonary artery pressure measurement and that obtained by echocardiography increased from 0.56 for Doppler signals obtained without UEAs to 0.75 with the injection of agitated 0.9% saline solution ($p < 0.001$). With a second-generation UEA, the correlation was 0.82 ($p < 0.001$) if injected into a peripheral vein. On Bland-Altman analysis, the agitated saline solution showed less variability (0.12 mm Hg) and narrower limits of agreement with the invasive measurement as compared to non-enhanced signals.[17]

One potential advantage of whole-circulation UEAs compared to exclusive enhancement of the pulmonary circulation with 0.9% agitated saline solution is the longer duration and stability of the Doppler signal.[18]

Furthermore, whole-circulation UEAs allow for the evaluation of valvular lesions on the left side of the heart. This technique has been of great value in the evaluation of patients with aortic stenosis, where obtaining transvalvular gradients can be challenging (Figure 2).

2.2. Whole-Circulation Ultrasound-Enhancing Agents Available on the Global Market

An ideal UEA should safely and effectively opacify, for a sufficient time, the cardiac chambers and their microcirculation. Modern diagnostic microbubbles consist of a capsule (lipid, protein, or polymer) containing a high-molecular-weight gas for stability (Figure 3).[1]

Typically, a few milliliters of a whole-circulation UEA contain around 2 billion microbubbles.

Second-generation (whole-circulation) UEAs currently commercially available on the worldwide market (Optison®, Definity®, SonoVue®), as well as the only extemporaneously compounded formulation, perfluorocarbon-exposed sonicated dextrose albumin (PESDA),[19] all consists of a high-molecular-weight gas encapsulated in albumin, chemical polymers, or phospholipids. In recent years, there has been active development of microbubbles by several pharmaceutical companies, with agents already commercially available and approved for clinical use in the United States, Asia, and Europe. In Brazil, only SonoVue® was available up to the time of this publication, marketed by Bracco Imaging do Brasil (Table 1).[1]

Optison® (GE Healthcare, Princeton, New Jersey) was the first perfluorocarbon (perflutren)-containing second-generation UEA approved for human use, indicated for improving left ventricular opacification after intravenous injection.

PESDA is an enhancing agent based on the perfluorocarbon gas decafluorobutane.[7] Rather than commercially available, it is institutionally custom-made by mixing dextrose, human serum albumin, and decafluorobutane in a 1:1:1 ratio, followed by manual agitation of the mixture in the syringe itself and subsequent direct sonication by removing the syringe plunger using a titanium rod that produces vibrations of 20,000 Hz for 90 seconds. Refrigeration below 4°C may be the best way to store PESDA for 48 hours.[19,20] PESDA microbubbles pass rapidly through the microcirculation and do not alter the left ventricular function, coronary or systemic hemodynamics, or pulmonary gas exchange.

Both PESDA and Optison® can produce consistent myocardial contrast in animal and human studies when used with new imaging modalities.

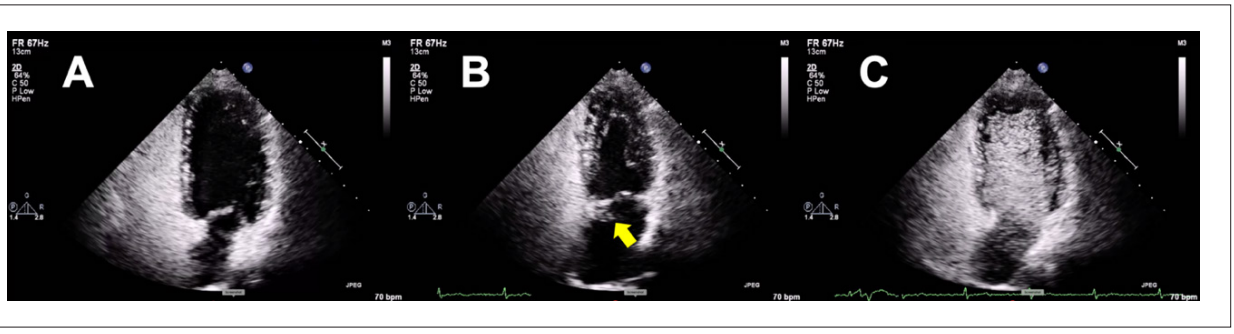


Figure 1 – *Apical 4-chamber view using second harmonic imaging with a high mechanical index (1.2) demonstrating (A) saline microbubbles reaching the right chambers 1 second after intravenous injection, (B) beginning of microbubble passage through the atrial septum in the second beat and (C) Valsalva maneuver revealing large passage of microbubbles (paradoxical shunt) to the left chambers.*

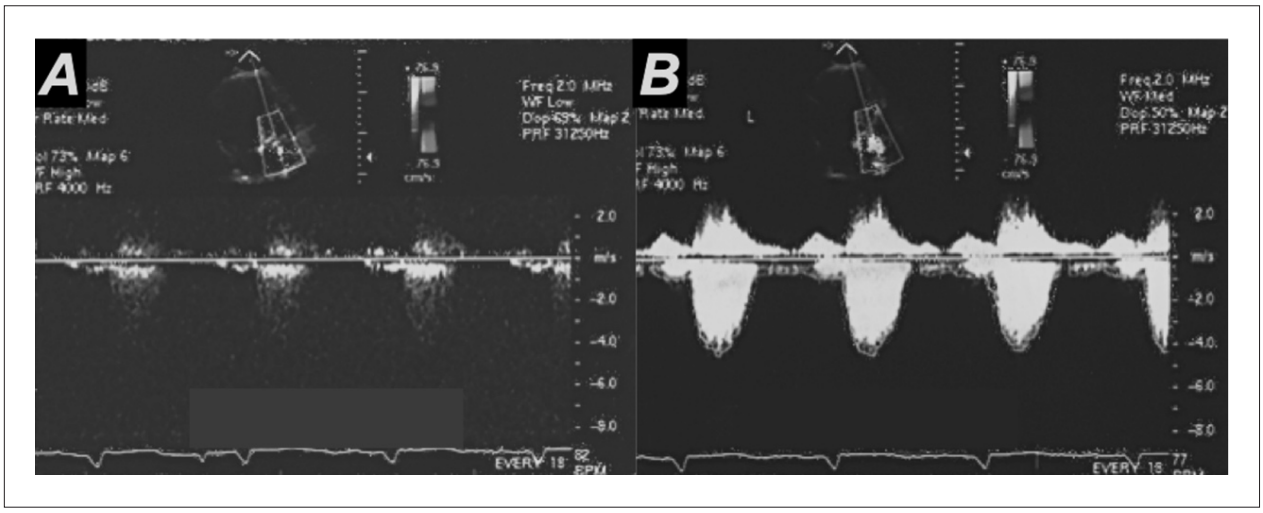


Figure 2 – *Image obtained in the apical plane demonstrating (A) a suboptimal continuous wave Doppler signal without UEAs in a 68-year-old male patient with a systolic murmur at the aortic focus. In (B), after administration of a whole-circulation UEA, there is a complete continuous-wave Doppler envelope demonstrating a peak systolic gradient of 76 mm Hg and a mean gradient of 41 mm Hg, consistent with significant aortic stenosis.*

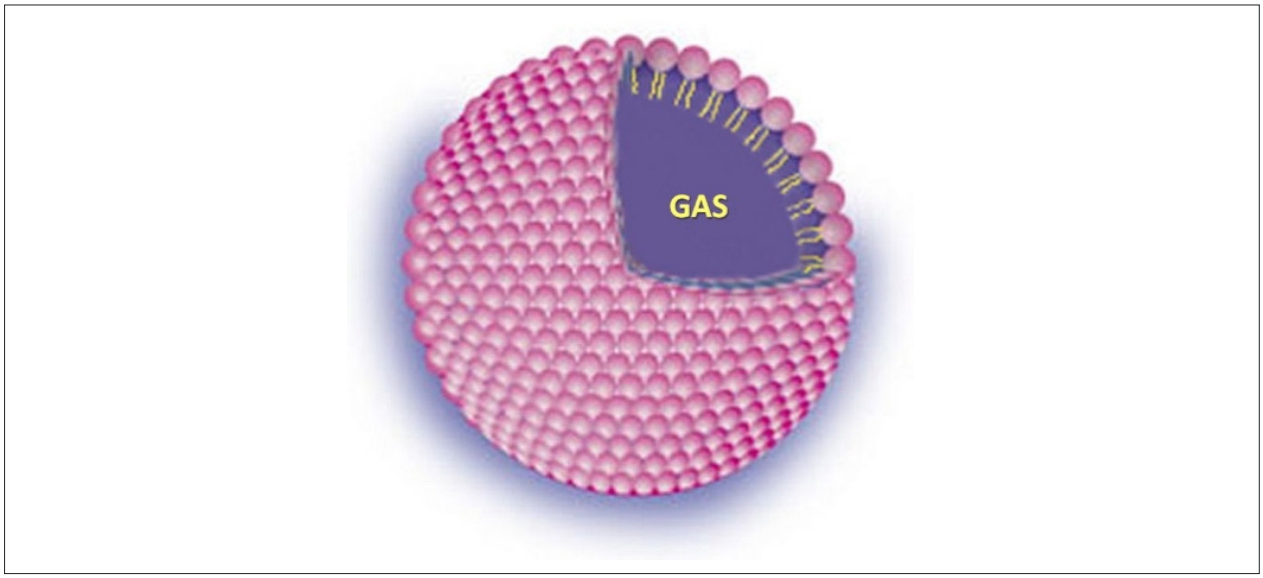


Figure 3 – *Example of a typical microbubble, consisting of a capsule with gas inside.*

Definity® (Lantheus Medical Imaging, North Billerica, Massachusetts) is also approved by the U.S Food and Drug Administration (FDA) for endocardial border detection. Definity® microbubbles consist of a capsule containing a long-chain lipid and a combination of saturated diacyl-phosphatidylcholine and polyethylene glycol surrounding a perflutren gas core. To create stable microbubbles, this mixture must be activated for 90 seconds with a proprietary device known as Vialmix®.

SonoVue® (Bracco Imaging, Milan, Italy) has been approved as a UEA in Europe, China, Brazil, Canada, and the USA (where it is branded as Lumason®). These microbubbles have a phospholipid capsule surrounding sulfur hexafluoride gas. Of the three main commercially available ultrasound-enhancing agents, only SonoVue® is available in Brazil up to the moment of publication.[1]

3. Implementation of Ultrasound-Enhancing Agents in Echocardiography Services

3.1. Equipment and Image Processing

Historically, the enhancing effects of UEAs when present in human circulation were observed with ultrasound equipment having extremely low resolution as compared to current devices.[1-5]

Table 1 – Characteristics of Ultrasound-Enhancing Agents Currently Used Worldwide

Manufacturer	Trade Name	Gas	Average Diameter (µm)	Molecular Weight (g/mol)	Capsule Composition
—	None (air microbubbles)	Ambient air (nitrogen [78%), oxygen [21%], other gases [1%))	100	O2: 14, N: 16	Not encapsulated (0.9% saline solution)
GE Healthcare	Optison®	Perflutren	3.9	188	Denatured albumin
Lantheus Medical Imaging	Definity® or Luminity®	Perflutren	1–3	188	Phospholipid / Lipids
Bracco Imaging	SonoVue®	Sulfur hexafluoride	2–8	146	Surfactant

1. EMEA Scientific Discussion. SonoVue, European Public Assessment-Report. 2004; http://www.emea.europa.eu/humandocs/Humans/EP-AR/SonoVue/SonoVue.htm. 2. GE Healthcare. Optison Prescribing Information. 2008; http://www.amershamhealth-us.com/optison/ 3. Luminity Summary of Product Characteristics. 2008). Perflutren (International Nonproprietary Name) is also known as perfluoropropane or octafluoropropane.

Several imaging modalities have been used for evaluation of the coronary microcirculation. For clinical practice purposes, several of these imaging methods are now of purely historical interest, such as intermittent harmonic imaging with a high mechanical index (MI), and will not be described herein.

An essential parameter in characterization of images acquired with the aid of UEAs is an index that measures the intensity of the acoustic energy released by the transducer, known as the MI, calculated by dividing the peak negative pressure by the square root of the center frequency of the transducer.

Early imaging methods utilized the stable cavitation effect using low mechanical indices (low acoustic energy), below 0.2, or inertial cavitation using high mechanical indices (high acoustic energy), above 0.8, as detailed below.

3.2. Real-Time Myocardial Perfusion Echocardiography Technology Imaging with low Mechanical Index

This is the imaging modality of choice for modern myocardial perfusion studies, due to its ease way of acquisition and standardization. Significant advances have been made with this technique, which can simultaneously assess myocardial perfusion and wall motion in real time with a low MI.

In this modality, real-time imaging of the heart is performed using an MI < 0.2, and intermittently, 10 to 15 simultaneous frames of high-energy acoustics with MI > 1.0 are applied in less than 300 ms (0.3 seconds), destroying all microbubbles in the acoustic beam and thus allowing subsequent observation of refill time and maximum plateau, two parameters which, when multiplied, provide a measure of microvascular flow (Figure 4).[23,24]

3.3. Myocardial Perfusion Analysis

Myocardial perfusion can be assessed qualitatively or quantitatively. Qualitative assessment of myocardial perfusion is based on the semi-quantitative visual determination of a graded scoring system as follows: 1 = normal (homogeneous and clear opacification), 2 = reduced (partial or heterogeneous opacification with normal wall thickness, "irregular pattern"), 3 = absent (no myocardial opacification). An enhancement score index can be calculated by dividing the sum of the enhancement scores given to each segment divided by the number of segments visualized (Figure 5).

Quantitative analysis is done in post-processing, using specialized software that enables quantification of various microvascular flow parameters. This is possible because microbubbles and red blood cells share similar kinetics; thus, by calculating the velocity and volume of microbubbles within the microcirculation, one can infer the velocity and volume of microvascular blood.

The method is based on a continuous, constant infusion of microbubbles until a stable peak microbubble plateau is reached in the myocardium. This plateau is proportional to the microvascular blood volume. Then, after destruction of microbubbles in the ultrasound field (bottom of Figure 4), one can multiply the peak plateau intensity, a parameter represented by the letter A, by the microvascular refilling rate (which is proportional to the blood flow velocity), represented by the Greek letter β, resulting in microvascular flow (A × β).

3.4. Artifacts in the Assessment of Myocardial Perfusion

For adequate assessment of myocardial perfusion, one must be able to distinguish possible artifacts from actual real perfusion defects. The most common artifact is attenuation of the ultrasound beam, which typically occurs in the basal segments in apical windows and can be distinguished from true defects by its location and spatiotemporal distribution. Attenuation typically affects not only the myocardium but also the adjacent epicardial and endocardial layers, and is usually present at rest and under stress, while induced perfusion defects are present only during stress and generally involve only the subendocardial layer throughout the cardiac cycle (Figure 6).

Another potential artifact is lung shadowing, which often masks the entire region, such as the anterior wall in the apical 2-chamber view. This artifact can be overcome by purposefully shortening this plane, which leads to shortening or exclusion of the apex but better delineation and enhancement of the middle and basal anterior wall segments. A third location where artifacts commonly occur is in the left ventricular apical region; if the proximal field gain is set too low, the apex may demonstrate a false (artifactual) perfusion defect.

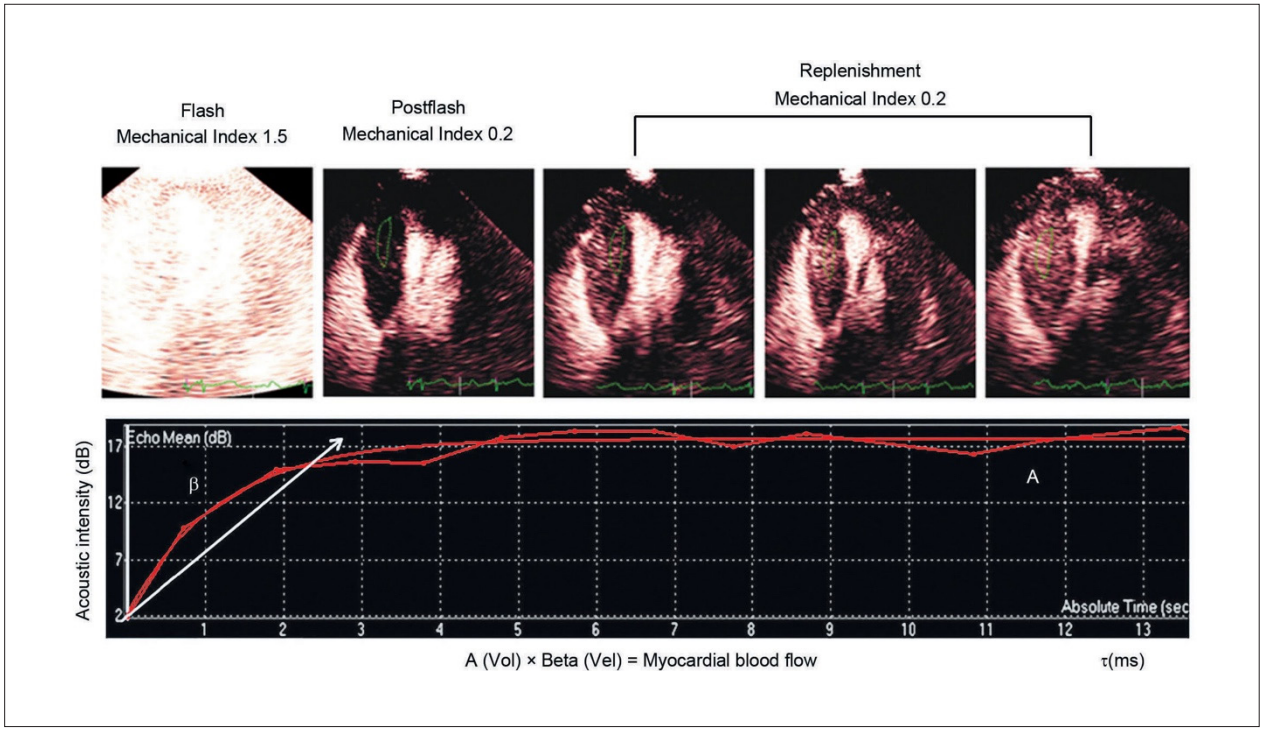


Figure 4 – *Visualization of myocardial refilling after microbubble destruction following high-intensity impulses (high mechanical index, > 1.0), known as flash. The software automatically calculated the peak acoustic intensity and the refilling velocity of each region of interest and generated time–intensity curves; when multiplied, these parameters correspond to microvascular flow. Sec: seconds; Vel: velocity; Vol: volume.*

Blooming artifacts also can occur immediately after the administration of a high UEA dose, causing strong signal intensity, exacerbating myocardial brightness, blurring the myocardial borders and obscuring possible perfusion defects. Blooming can be eliminated by reducing the gain, reducing the concentration of UEAs by reducing the speed of infusion, or simply waiting for a few cardiac cycles until the concentration of the agent in the blood is naturally reduced (Figure 7).

Finally, attenuation artifact, which can be confused with perfusion defects, is evident mainly in the basal segments in apical projection (Figure 8).

3.5. Equipment Configuration and Settings

Most echocardiography equipment vendors have presets for imaging with very low MI, which ensures that optimal pulse sequence schemes are being used to adequately detect UEAs. The ideal MI is the lowest MI that provides strong enhancement with sufficient penetration (no attenuation) and minimizes background noise. The ideal MI varies according to the patient's acoustic window and the central frequency chosen for the transducer.

Multi-pulse nonlinear fundamental imaging is preferable to second harmonic imaging, and a very low MI < 0.2 will reduce microbubble destruction and improve enhancement as compared to second harmonic imaging. As a rule, higher frequencies provide lower penetration but higher image resolution. Conversely, patients with a larger body surface area (BSA) may require a relatively higher MI and/or lower frequencies.

The focus must be positioned distally in the acoustic beam to avoid premature destruction of the UEAs; it is generally positioned below the mitral annulus plane in an apical window. In certain circumstances, the focus can be moved to the near field to better visualize an apical thrombus, ventricular apex morphology, and wall motion or perfusion.

3.6. General Principles: Dosage and Administration

Optimal dosage often represents a compromise between attenuation (indicating that the dose is too high) and UEA effect duration (which may be too short if the dose is too low). UEA injection speed can also impact image quality; faster UEA injection and saline flush rates or rapid continuous infusion may create higher concentrations of microbubbles, resulting in basal attenuation artifacts and blooming in the proximal ultrasound field, while slower injection rates may result in swirl artifacts, with incomplete opacification of the left ventricular apical segments.

Bolus administration generally reduces imaging time, but due to higher concentrations of UEAs, attenuation artifacts may be observed. Bolus doses of UEAs are recommended with slow injections of saline flush of 5 to 10 mL in the adult population, infused over a period of at least 5 seconds for each projection studied.

Alternatively, continuous infusions provide longer, more consistent enhancement with fewer attenuation artifacts and may

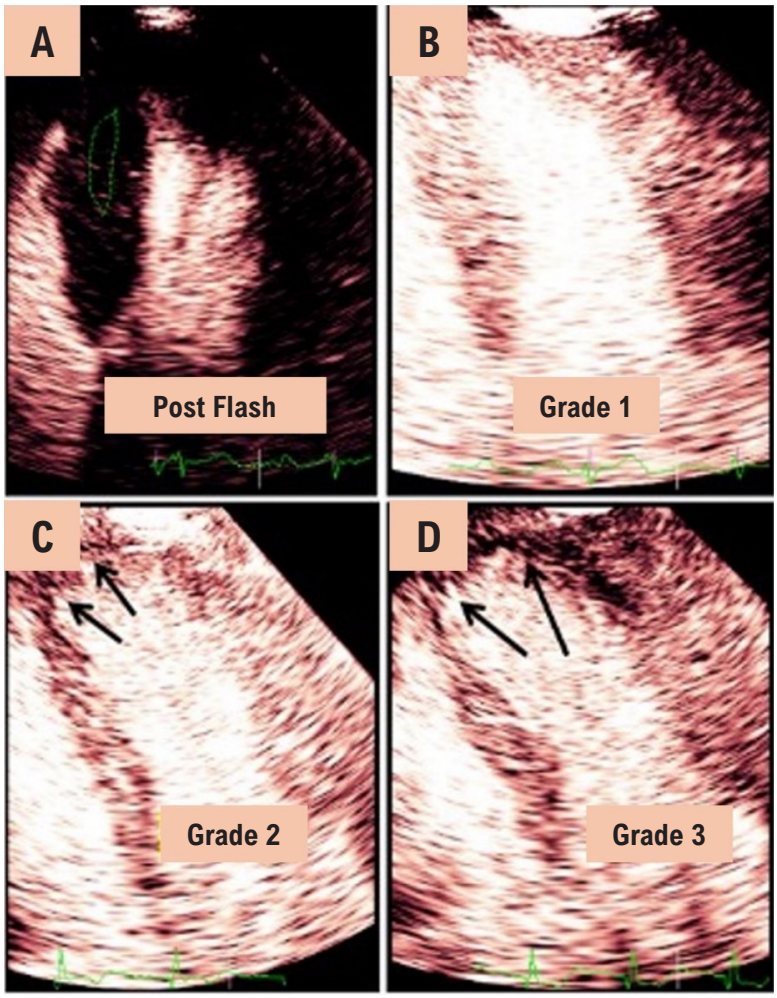


Figure 5 – *Examples of visual determination of postflash myocardial refilling by microbubbles for the calculation of myocardial perfusion scores. (A) All evidence of microbubbles in the myocardium is cleared postflash. (B) Baseline homogeneous opacification of the myocardium (score 1). (C) Heterogeneous reduction with a subendocardial perfusion defect in the apical septal region of the left ventricle (arrows) during low-dose dobutamine infusion (score 2). (D) Absence of opacification, with a transmural perfusion defect in the apical region of the left ventricle (arrows) at peak dobutamine stress (score 3).*

allow individual optimization of enhancement by adjusting the infusion rate during imaging. Although both bolus injections and continuous infusions can be used to assess myocardial perfusion, continuous infusion allows for better quantification of myocardial blood flow abnormalities by using the real-time myocardial perfusion echocardiography (RTMPE) imaging.

A 20-gauge cannula or larger is recommended because UEAs can be degraded by a small-bore intravenous cannula. Cannulation of an antecubital or central vein is preferable because the site of UEA delivery should ideally be a large vein as close to the heart as possible. Using a 3-way stopcock, with the enhancing agent always connected in a straight line, minimizes the risk of premature microbubble destruction. The saline solution for flushing must be connected to the side T port.

3.7. Protocol: Bolus Administration during Rest or Under Cardiovascular Stress

- Adjust the initial dose by bolus administration, always followed by 5 to 10 mL of saline flush (0.9% NaCl), starting with the lowest recommended dose and adjusting as needed until complete left ventricular opacification (LVO) is achieved, while avoiding attenuation of the basal LV segments.
- For pharmacological stress studies, images should be acquired at baseline, at low dose, at peak stress, and during the recovery phase, as per recent guidelines.[25]
- For studies under physical exercise stress (e.g., treadmill tests), the UEA bolus and flush should be administered at rest and immediately (approximately 30 seconds) before the end of exercise. When the examination is performed on a supine



Figure 6 – *Example of subendocardial (lower arrow) and transmural (upper arrow) perfusion defects in a patient with an anteroseptal/apical acute myocardial infarction.*

or in-bed cycle ergometer, an extra bolus can be performed during exercise to allow for contractility and myocardial perfusion at peak or immediate post exercise.

• A simple and empirical strategy for images acquired during and after physical exertion, due to the high cardiac output state, is to forego saline flush by injecting the UEA at peak (or immediately after the end of) exercise and, when the patient is in the left lateral decubitus position with a peripheral IV in the right upper limb, simply elevate the patient's right arm to 90° for a few seconds. This allows drainage of most of the microbubbles trapped in the peripheral venous system, working very similarly to a saline flush.

• Avoid applying strong pressure when infusing or injecting UEAs, as this can destroy the microbubbles.

• ConFigure the ultrasound system to use enhancement presets with non-linear multi-pulse imaging, at fundamental frequency with amplitude modulation, or combining amplitude/phase modulation (RTMPE) with a low MI ≤ 0.2.

• Alternatively, low-mechanical-index (0.2–0.3) second harmonic images can be used, but they do not allow for adequate analysis of myocardial perfusion, although they may be ideal for delineating endocardial borders and, therefore, left ventricular opacification (LVO).

3.8. Tips for Stress Image Acquisition

For left ventricular opacification: homogeneous enhancement of the left ventricular cavity is observed, without turbulence or blooming at the apex or shadowing or acoustic attenuation in the basal segments in the apical 2-chamber plane.

Near- and distal-field time gain compensation adjustments may be necessary, and the transmission focus may need to be adjusted to the center of the left atrial cavity in the apical 4-chamber plane. Once these adjustments are made, they must be kept constant throughout the duration of the study, both at rest and under any form of stress. Newer platforms have already incorporated these adjustments to the proximal field.

For perfusion analysis: proceed as above, with the additional step of using a high IM pulse (flash), generally containing 10 to 15 frames, which aims to destroy all microbubbles within the acoustic plane, to allow observation (and documentation for later analysis) of the microcirculation refilling time.

With current two-dimensional transducers, normal myocardial perfusion is indicated by complete myocardial enhancement within 5 seconds post flash at rest and within 2 seconds post flash under stress conditions. Abnormal myocardial perfusion is indicated by delayed or absent refilling or by a post flash distribution

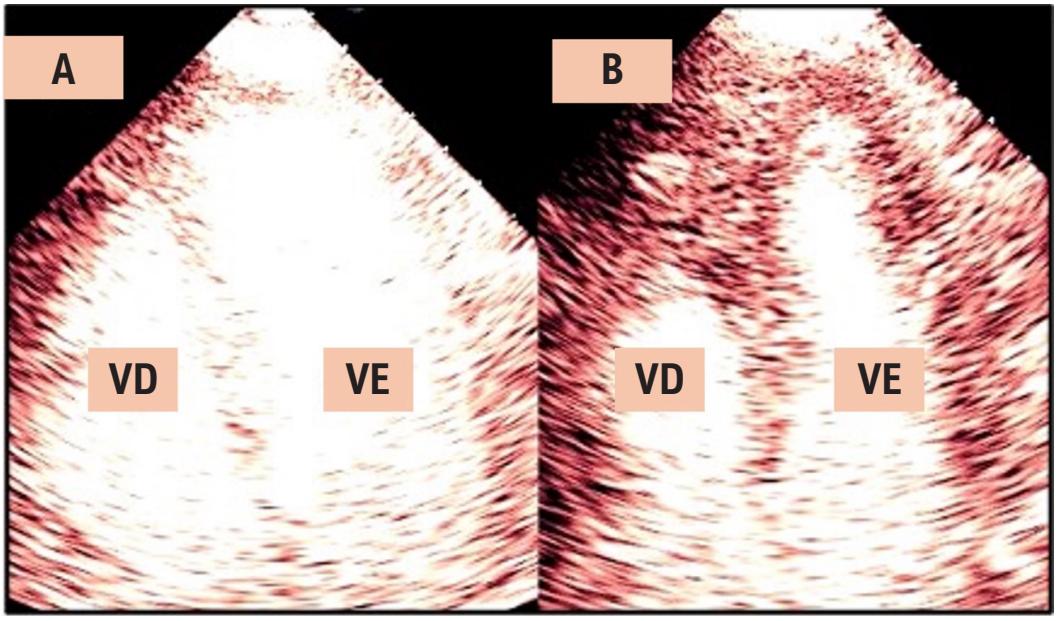


Figure 7 – *Example of blooming in apical 4-chamber view. In (A) the strong signal intensity exacerbates image brightness in the apical region, making it difficult to differentiate the cavity from the myocardium. In (B), the ultrasound-enhancing agent infusion has been reduced, allowing acquisition of an optimal image. LV: left ventricle; RV: right ventricle.*

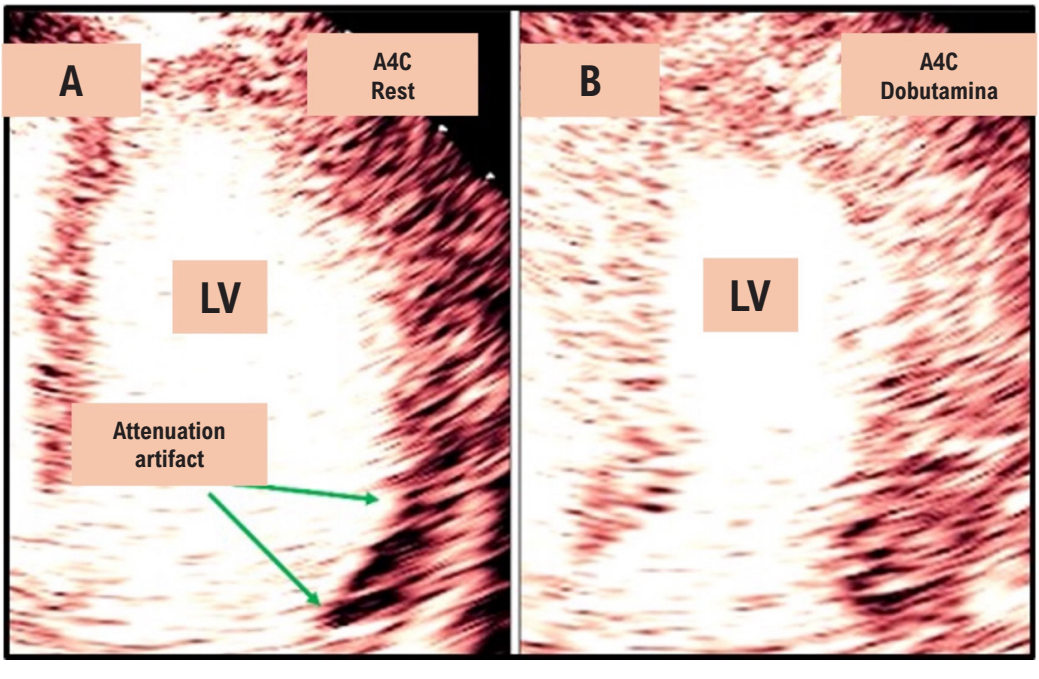


Figure 8 – *Example of attenuation artifact. (A) Apical 4-chamber view demonstrating a perfusion pseudo-defect along the entire length of the basal segment (green arrows) of the anterolateral wall. (B) During real-time myocardial perfusion echocardiography imaging under dobutamine stress, a more homogeneous perfusion is observed by simply bringing the anterolateral wall to the center of the ultrasound beam, corroborating the hypothesis of a pseudo-defect. A4C: apical 4-chamber; LV: left ventricle.*

of UEAs which is limited to the subendocardial layer (mild ischemia) or transmural layer (extensive ischemia).

Myocardial perfusion analysis consists of two elements: peak blood volume and capillary refill velocity, which is equivalent to the speed of microvascular blood flow. The absence of blood volume at peak enhancement may indicate the presence of myocardial infarction or severely reduced microvascular perfusion. Furthermore, delayed restoration of blood flow after microbubble destruction occurs due to reduced myocardial blood flow and may indicate hemodynamically significant epicardial coronary stenosis and/or a compromised microcirculation. Since the interpretation of microvascular physiology is complex, myocardial perfusion imaging should be combined with wall motion analysis to provide information that allows for the proper interpretation of various possible scenarios involving the state of the microcirculation and wall motion, including normal or abnormal conditions such as ischemia, stunning, hibernation, and myocardial necrosis. During stress studies, myocardial perfusion imaging is the most sensitive parameter and can detect patients at low risk for cardiovascular events, while wall motion analysis is a more specific parameter and can rule out higher-risk patients; its prognostic value has been validated in numerous clinical studies.[26-38]

Image planes can be adjusted to bring the myocardial segments of interest closer to the center of the image plane, to enhance microbubble detection accuracy (Table 2, Figure 9, and Supplementary Video 1).

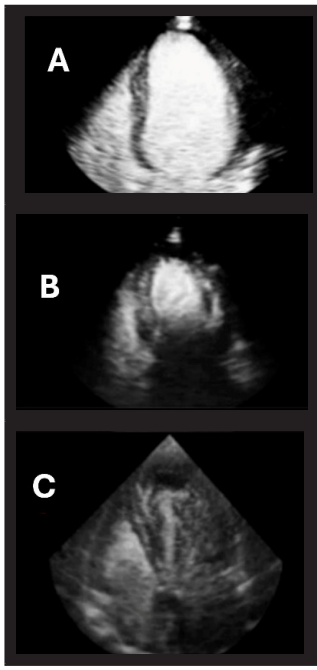
4. Clinical Applications in Resting Transthoracic Echocardiography

4.1. Ventricular Opacification, Measurement of Left Ventricular Volumes and Ejection Fraction

Opacification of the cardiac chambers is the main indication for clinical use of UEAs, as it allows real-time visualization of left ventricular motility and measurement of left ventricular volumes and ejection fraction.

Table 2 – Recommendations for preset adjustments

Use a non-linear fundamental image transducer preset (generally 1.6 to 2.0 MHz)
Use a very low mechanical index (≤ 0.2)
Position the focus at or slightly below the mitral annulus in the apical 4-chamber view
Adjust the time gain compensation sliders for higher gain in the proximal field, if necessary
Maintain the frame rate around 30 fps



Ideal opacification	Ideal administration	
Attenuation - Dark acoustic shadow obstructing the view of the entire or significant part of mid and distal acoustic field	**Cause** - Rapid infusion rate or UEA dose is too high	**Correction** - Wait until UEAs are cleared - Reduce infusion rate or volume - transient increase MI to deplete microbubbles within the LV cavity
Swirling - inadequate LV opacification	**Cause** - Slow administration rate _ MI is too high or focus is positioned above the level of mitral anulus position - reduced LV function	**Correction** - Increase dose and rate of administration - Reduced MI - Adust the focus: it should be below the mitral annulus

Figure 9 – *Demonstrating the ideal opacification conditions (A), attenuation (B) and apical swirl (C). LV: left ventricular; MI: mechanical index; UEA: ultrasound-enhancing agents.*

Left ventricular volumes should be obtained by tracing at the interface between compacted and trabecular myocardium, as recommended in the latest American and European societies' guidelines for the quantification of cardiac chambers. It is recommended as standard that left ventricular volumes be quantified using Simpson's biplanar method, which results in a very close correlation to cardiovascular magnetic resonance imaging (Figure 10).[39-41]

Several studies comparing two-dimensional echocardiography with and without UEAs, using CMR as the reference standard, have demonstrated greater accuracy in determining volumes and LVEF with the use of UEAs. Furthermore, inter- and intra-observer variability was shown to be much lower with UEAs. Although three-dimensional (3D) echocardiography has improved the reproducibility and reliability of serial LVEF measurements, such as in patients undergoing chemotherapy, the use of UEAs is simpler and more readily available than 3D echocardiography at most facilities, and its accuracy and ability to improve the quality of image interpretation have been validated in various clinical settings.[15-18,21,22,42,43]

The analysis of regional wall motion abnormalities (RWMA) is subjective and is significantly limited by large interobserver variability, even when using high-quality images.

There is no gold standard for this subjective assessment, and it depends on image quality, highlighting the importance of accurately detecting the endocardium and epicardium, since the assessment of RWMA depends on wall thickening. Studies have addressed the interobserver variability of echocardiography with and without UEAs compared to CMR and ventriculography and have demonstrated a high correlation with CMR.[40,41,43]

Thus, in addition to improving left ventricular volumetric assessment (14-24), UEAs can improve the prediction of cardiovascular outcomes beyond clinical assessment and LVEF measurement without the use of UEAs.[44]

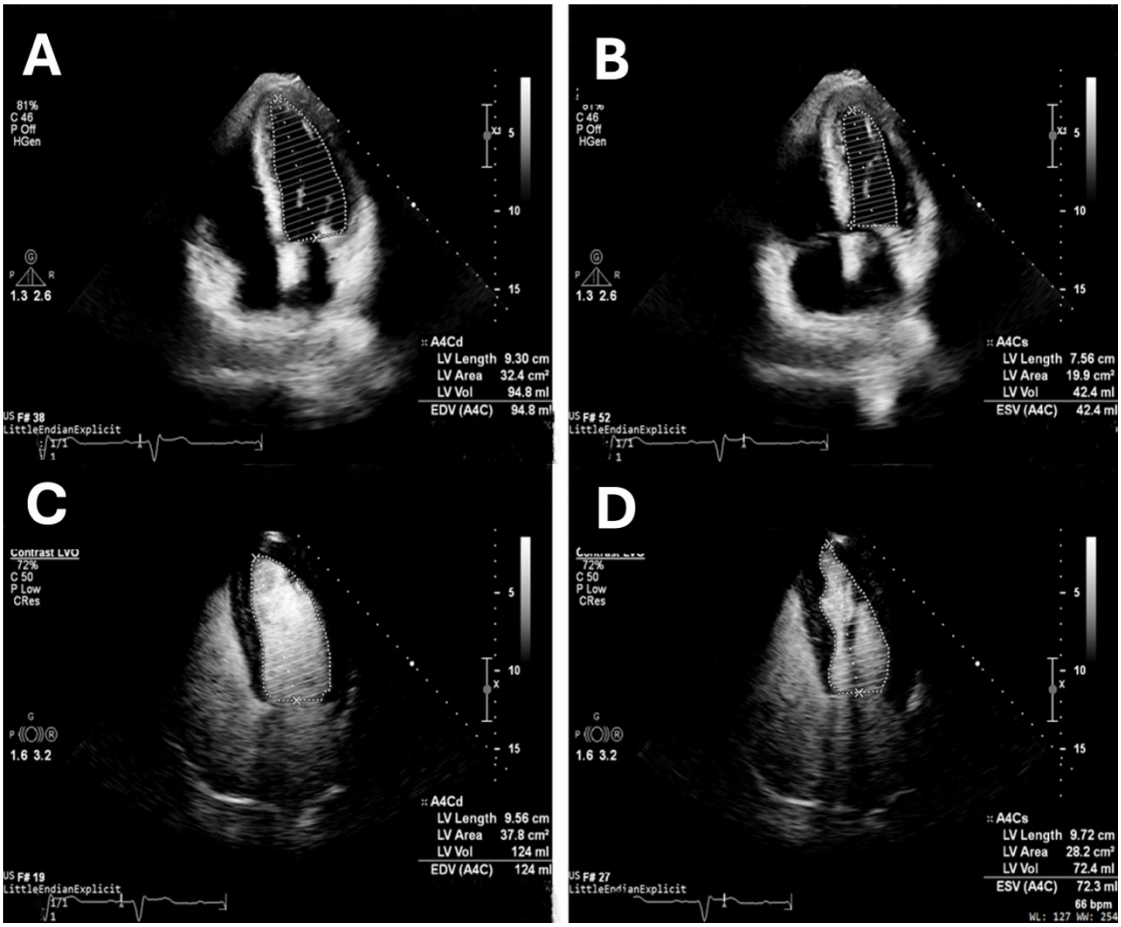


Figure 10 – *Differences in end-diastolic and end-systolic volumes observed in the same patient without ultrasound-enhancing agents (top) and with ultrasound-enhancing agents (UEAs) and low MI images (bottom). Top row, left to right: (A) Quantification without UEAs of left ventricular end-diastolic volume (94.8 mL) and (B) end-systolic volume (42.4 mL). Bottom row, left to right: (C) Quantification with UEAs of left ventricle end diastolic volume (124 mL) and (C) end-systolic volume (72.3 mL).*

Some conditions constitute established indications for the use of UEAs, such as investigation of a cardiac source of embolism, differential diagnosis of intracardiac thrombi versus masses, apical hypertrophic cardiomyopathy, LV noncompaction, and hypereosinophilic heart disease (Loeffler's syndrome).

4.2. Intracardiac Thrombi

Intracardiac thrombi carry potentially serious clinical repercussions, including systemic embolization. Similarly, inadequate treatment with antithrombotic and anticoagulant agents can also expose the patient to significant risk, and such therapy should therefore be based on imaging findings obtained after opacification of the cardiac chambers.

Furthermore, myocardial perfusion imaging allows for the evaluation of the tissue characteristics of ventricular masses, differentiating thrombi, which are avascular, from tumors, and allowing for a more accurate echocardiographic diagnosis with precision similar to that of CMR.[45,46] (Figure 11 and Supplementary Video 2).

A study using transesophageal echocardiography in 180 patients with atrial fibrillation before electrical cardioversion[47] found atrial thrombi in 14 (15.6%) patients with the use of UEAs and in 10 (11.1%) of those without UEAs ($p < 0.001$). Thrombi could be ruled out in 60 (66.7%) patients during TEE without UEAs and in 75 patients (83.3%) during TEE with UEAs ($p < 0.01$).

Electrical cardioversion was performed subsequently, after thrombi had been ruled out and at the investigator's discretion. Among patients who received UEAs, 74 (82.2%) were cardioverted, and none experienced a cardiovascular complication ($p = 0.084$); of those imaged without UEAs ($n = 76$, 84.4%), 3 suffered thromboembolic complications after electrical cardioversion.

4.3. Intracardiac Masses

Intracardiac masses can be normal structures, such as false tendons, accessory papillary musculature, and exuberant trabeculation, or they can be pathological, such as thrombi, vegetations, or tumors. Two-dimensional echocardiography is the most widely used diagnostic imaging modality in the initial evaluation of cardiac masses, as it offers real-time assessment with high spatial and temporal resolution. Despite the development of several echocardiographic criteria for such assessment, diagnostic errors and misclassifications can still lead to unnecessary surgeries or anticoagulation.[9,45]

Thus, in the presence of a suspicious cardiac mass that is not adequately evaluated by two-dimensional echocardiography, the use of UEAs allows for better characterization of the structure, potentially confirming or refuting the diagnostic hypothesis and avoiding diagnostic errors.

Malignant tumors are masses with abnormal neovascularization, which supplies the rapidly growing tumor cells, while benign tumors are generally poorly vascularized and exhibit perfusion resembling that of the myocardium. Thrombi, on the other hand, are avascular structures. Therefore, echocardiographic imaging with the aid of UEAs and RTMPE technology allows for the characterization of vascularization and, therefore, differentiation of cardiac masses[45] (Figure 12).

This characterization is performed both qualitatively and quantitatively by comparing the perfusion levels of various types of cardiac masses with that of the adjacent myocardium. The qualitative approach is based on the visual analysis of the post flash refill time of the UEA, and categorized as absence of enhancement, partial or incomplete enhancement, and complete enhancement. Complete enhancement or hyperenhancement of a mass compared to the surrounding myocardium supports the hypothesis that it is a highly vascularized tumor, which increases clinical suspicion of

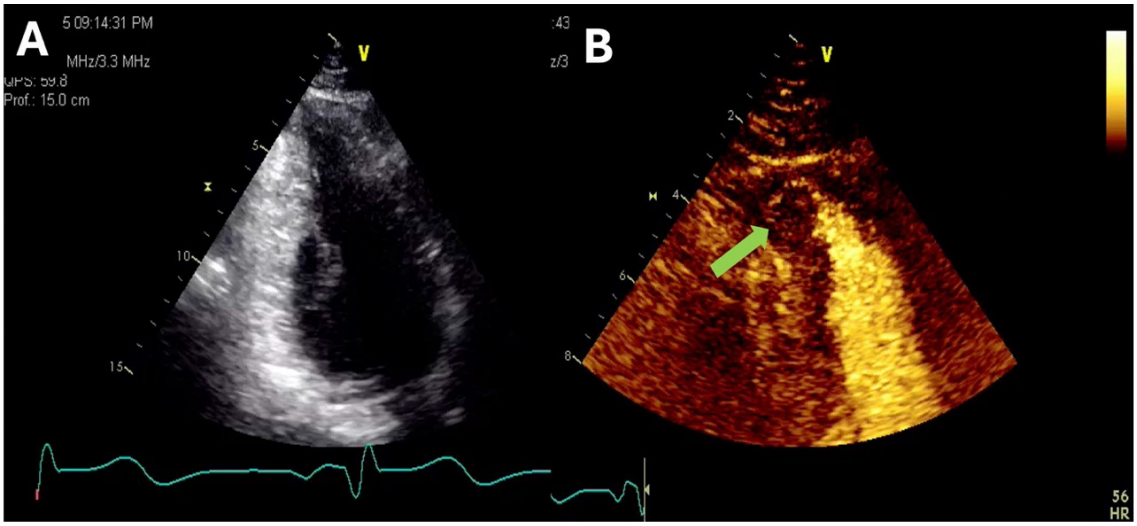


Figure 11 – *Apical 2-chamber view demonstrating (A) the impossibility of visualizing the inferior lateral wall and apex. In (B), in addition to adequate delineation of the previously non-visualized segments, an apical thrombus can be seen.*

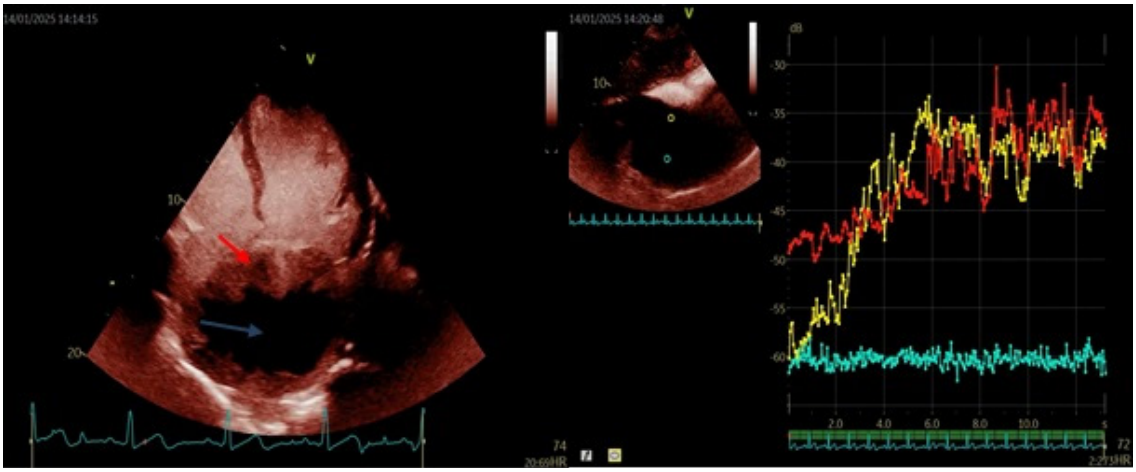


Figure 12 – *Left: transthoracic echocardiogram with real-time myocardial perfusion technology, in the apical 4-chamber view, showing a mass within the left atrium. This is a metastatic paraganglioma. This mass shows peripheral uptake of ultrasound-enhancing agents (UEAs) (red arrow), without central uptake, due to central tumor necrosis (blue arrow), confirmed by histopathological examination after surgical resection. Right: quantitative perfusion assessment with UEAs, demonstrating uptake in the peripheral portion of the tumor (yellow), compared to hepatic uptake (red) and absence of perfusion in the center of the mass (blue).*

a malignant mass. Stromal tumors, such as myxomas and fibroelastomas, generally are poorly vascularized and therefore show partial or imperceptible enhancement (Figure 13), while thrombi are avascular and consequently do not show enhancement.[45,48]

4.4. Myocardial Diseases

In apical hypertrophic cardiomyopathy, enhanced studies allow identification of the characteristic "ace of spades" shape of the left ventricular apex during systole (Figure 14), differentiating it from other common conditions affecting the left ventricular apex such as apical thrombi, aneurysms, or endomyocardial fibrosis (Figures 14 and 15 and Supplementary Videos 4 and 5) and also finding complications thereof, such as apical aneurysm and the presence of thrombi within (Figure 16 and Supplementary Video 6).[9,11,49,50]

Noncompaction cardiomyopathy, also known as left ventricular noncompaction, is an uncommon yet increasingly recognized abnormality that can lead to heart failure, arrhythmias, cardioembolic events and death. It results from alterations in the myocardial structure, which consists of two layers: a thin, compacted subepicardial myocardium and a thicker, noncompacted subendocardial myocardium. Two-dimensional echocardiography may be insufficient to identify the excessive trabeculation characteristic of this disease. In this scenario, ultrasound-enhanced echocardiography allows for adequate visualization of this excessive trabeculation. Using a slightly higher MI, for example 0.3 to 0.4, one can improve the evaluation of trabeculations by minimizing the destruction of low-velocity microbubbles located within the trabeculated myocardium, while maintaining adequate resolution of the ventricular myocardium[51] (Figure 17).

4.5. Complications of Acute Myocardial Infarction and Use in the Emergency Setting

Left ventricular aneurysm is an often an asymptomatic complication of a previous infarction. True aneurysms are localized, characterized by thin walls, usually dyskinetic and with apical dilation and generally involve the entire thickness of the ventricular wall. These findings are usually easily visualized on transthoracic echocardiography (TTE). However, if the apical region is not adequately visualized, an aneurysm may go unnoticed until the study is supplemented with an UEA.

Other complications, such as pseudoaneurysm, free wall rupture, and ventricular septal defects, are life-threatening and can usually be detected by echocardiography without UEAs. However, acoustic windows may be inadequate due to anatomy, patient position, and clinical conditions (e.g., being in the supine position and intubated) that prevent an ideal view of the apex. Under these conditions, UEAs can be essential in establishing the diagnosis, as well as in detecting other associated complications, such as thrombi.

About 10 to 15% of TTEs obtained in intensive care unit (ICU) settings are technically limited – up to 30% in patients who are under invasive ventilatory support, bedbound, and have dressings over areas of acoustic windows. It is precisely in these scenarios that speed and accuracy in cardiovascular assessment can determine clinical outcomes.[52]

The safety of UEAs in critical patients is well established; two studies were decisive in this context. The first,[53] a retrospective comparative analysis of 2,900 critically ill patients who underwent echocardiography with UEAs compared to 11,600 without enhancing agents, found no significant difference in short-term mortality (OR 1.18; 95% CI 0.82–1.71; p = 0.37). The second,

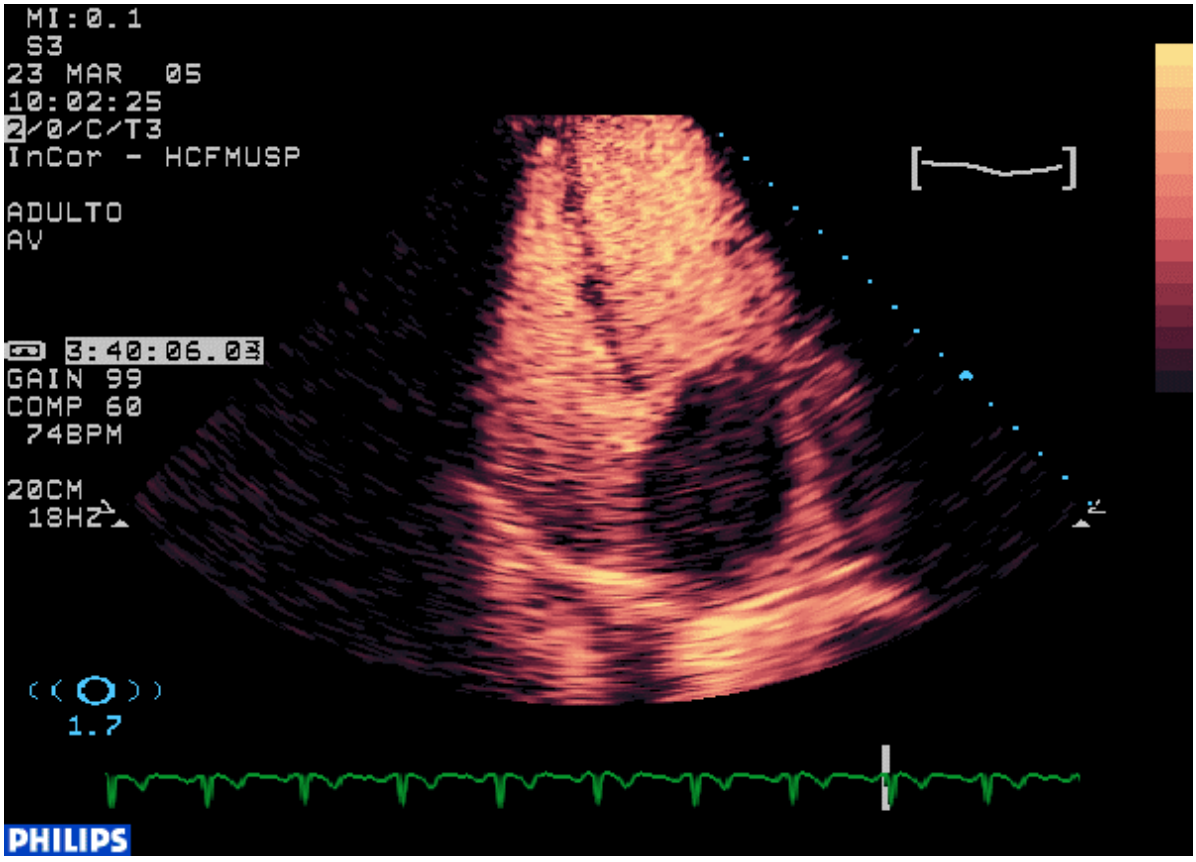


Figure 13 – *Transthoracic echocardiogram with real-time myocardial perfusion technology, in the apical 4-chamber view, showing a mass within the left atrium. This is a left atrial myxoma.*

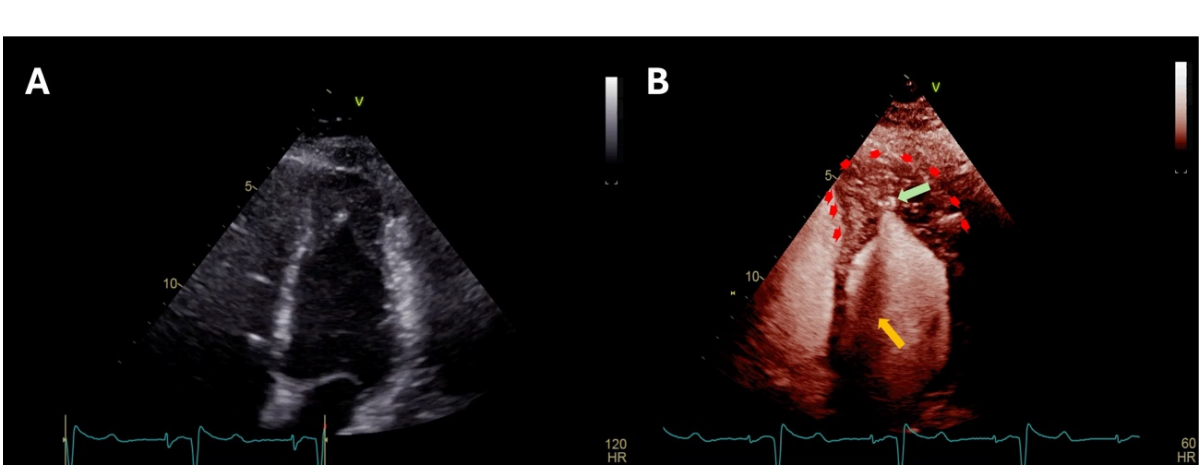


Figure 14 – *Example of apical hypertrophic cardiomyopathy (Yamaguchi disease): side-by-side images (A) without and (B) with ultrasound-enhancing agents (UEAs) and real-time myocardial perfusion echocardiography imaging technology. Note that in addition to the UEA-enhanced image better delineating the areas of hypertrophy (red arrows), it also demonstrates the presence of a small calcified apical thrombus (green arrow), confirmed by the presence of strong acoustic shadowing (yellow arrow).*

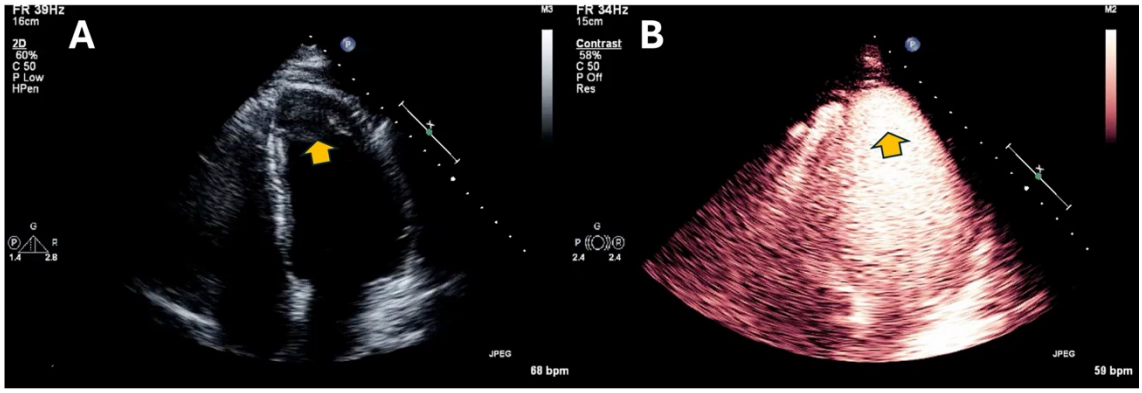


Figure 15 – *Echocardiography using ultrasound-enhancing agents (UEAs) clearly shows an apical aneurysm (A) in a patient with a history of anterior wall myocardial infarction 2 years before. The image on the left (A) raises the suspicion of apical thrombi (yellow arrow), which are ruled out after the injection of UEAs, as seen in (B) (yellow arrow). The purported thrombus in the non-enhanced image (A) actually corresponds to a near-field artifact caused by ring-down phenomena (resonance).*

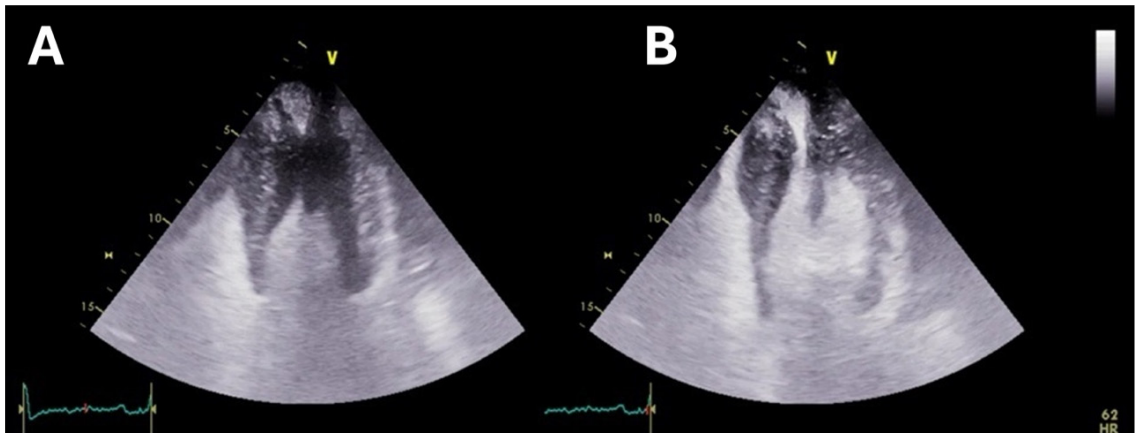


Figure 16 – *Midventricular hypertrophic cardiomyopathy with apical aneurysm and no thrombus, well visualized with the aid of ultrasound-enhancing agents both in systole (A) and in diastole (B).*

a broader study with a greater impact on this scenario, involved 16,217 ICU patients who underwent echocardiography with UEAs, matched with an equal number imaged without UEAs, and showed lower 48-hour mortality in the UEA group (1.7% vs 2.5%; OR 0.66; 95% CI 0.54–0.80), with a relative risk reduction of 34%.[54] As a likely explanation, the higher image quality obtained with the use of UEAs leads to faster and more accurate diagnoses, which in turn allows more appropriate clinical decision-making.

In a prospective, controlled study of 632 consecutive patients, 28% of whom were admitted to the ICU, the use of UEAs was associated with "rescue" of 83% of examinations with limited windows. These results were also associated with a cost reduction of $122 per patient.[55] In an expanded cohort evaluating echocardiograms with and without the use of UEAs in 1,538,864 million patients in ICUs across 773 hospitals, a 30% reduction in the need for repeat transthoracic echocardiograms (TTEs) and a 10% decrease in the mean ICU length of stay (4.15 days with UEAs vs 4.59 days without UEAs; $p < 0.0001$) were demonstrated.[56]

Diagnostic evaluation and risk stratification of patients presenting with chest pain in emergency departments is challenging. In up to 40% of cases, the initial electrocardiogram is not diagnostic, and biomarkers can take hours to change. Therefore, a significant proportion of patients are hospitalized, but only 10-30% have a confirmed diagnosis of acute myocardial infarction or acute coronary syndrome. Given these limitations, echocardiography with UEAs allows for the combined assessment of regional function and myocardial perfusion, providing immediate information to support diagnosis and risk stratification.[57,58]

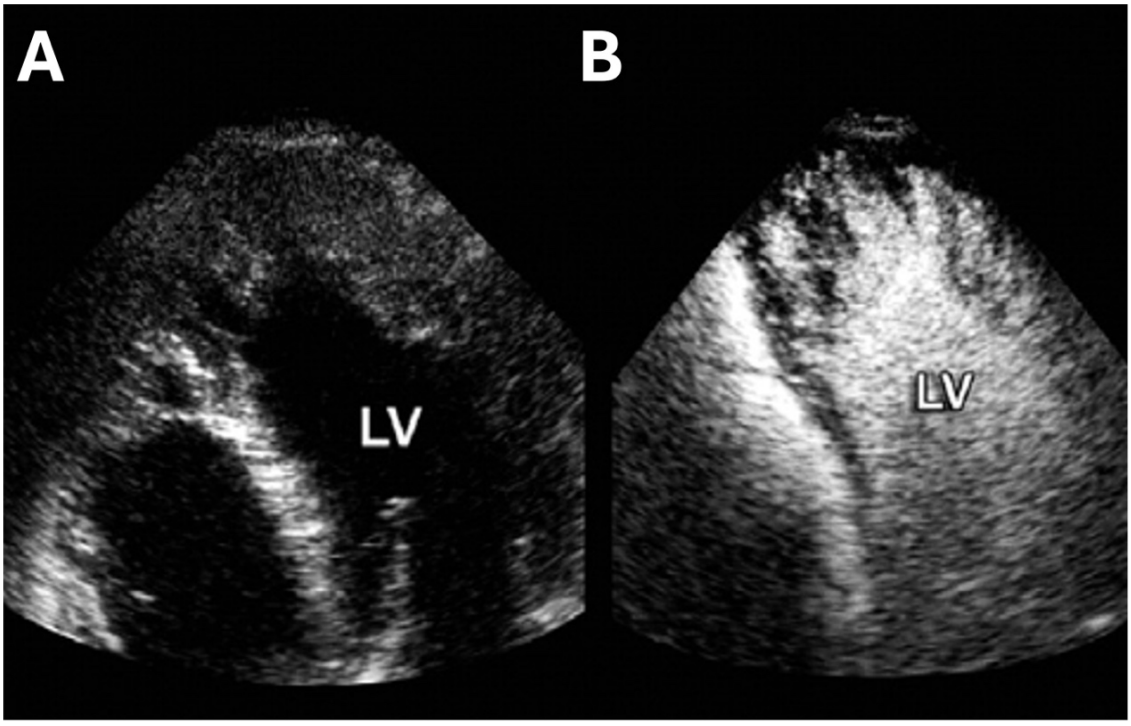


Figure 17 – *Two-dimensional echocardiogram in apical 4-chamber view demonstrating excessive apical trabeculations: (A) trabeculations are difficult to visualize in non-enhanced imaging and (B) trabeculations clearly evidenced by the use of UEA. LV: left ventricle.*

In a study evaluating 1,017 patients[58] who presented to the emergency department with chest pain and absence of ST-segment elevation, echocardiography with UEAs was performed to analyze left ventricular perfusion and wall motion. Overall, 16.3% of these patients experienced early cardiac events (cardiac death, MI, ACS, heart failure, and myocardial revascularization) within the first 48 hours. The addition of myocardial perfusion assessment by UEAs to the clinical and electrocardiographic model significantly increased the prognostic power for early events ($p < 0.0001$), and the inclusion of wall motion provided additional value ($p = 0.0002$). Patients with abnormal perfusion had a 6.1-fold higher risk (95% CI 3.5–10.6) of early events than those with normal perfusion. Patients with abnormal wall motion had a 2.4-fold higher risk (95% CI 1.5–3.7) than those with normal wall motion, and the odds of early events were 14.3-fold higher (95% CI 8.3–24.8; $p < 0.0001$) when both were abnormal. At a median follow-up of 7.7 months, 28.7% of patients had experienced cardiac events, and again, the analysis of perfusion and wall motion added incremental prognostic value ($p < 0.0001$). In another study, evaluating 957 patients with chest pain and a nondiagnostic ECG in the emergency department, the use of echocardiography with UEAs identified 523 patients with normal imaging (normal perfusion and wall motion), who had a low rate of cardiac events in the first 24 hours (0.6%), increasing the safety for decision-making related to early hospital discharge and avoiding unnecessary hospitalizations. In a decision simulation model, incorporating combined perfusion and wall motion assessment by echocardiography with UEAs had the potential to decrease hospitalizations by 45%, with a cost reduction of $900 per patient.[59]

4.6. Right Ventricular Evaluation

Although UEAs exclusive to the pulmonary circulation (agitated saline solution microbubbles ~100 μm in diameter) can be used to visualize abnormalities in the cardiac chambers connected to the pulmonary vasculature, the effect of this enhancement is short-lived. When a more lasting enhancement or better differentiation of the endocardial borders of the right ventricle is needed, whole-circulation UEAs (~2 μm) can be used instead, which allows analysis of morphology, function and microvascular perfusion, including changes in wall motion, detection of cardiac tumors and thrombi, as well as the characterization of normal structures, such as prominent trabeculations or the moderator band. In these scenarios, modified parasternal and apical windows should be used in combination for adequate analysis.[60]

4.7. Recommendations for use of Ultrasound-Enhancing Agents in Transthoracic Echocardiography

Recommendations	Strength of recommendation	Certainty of evidence
The use of transthoracic echocardiography with UEAs should be considered when two or more contiguous left ventricular segments are inadequately visualized	STRONG	HIGH
The use of transthoracic echocardiography with UEAs should be considered for the detection and differential diagnosis of intracardiac thrombi and masses	STRONG	MODERATE
The use of transthoracic echocardiography with UEAs should be considered when two or more contiguous left ventricular segments are inadequately visualized and clinical decision-making depends on accurate measurements of left ventricular volumes and ejection fraction, particularly in patients receiving potentially cardiotoxic therapies or being evaluated for implantation of an implantable cardioverter-defibrillator or cardiac resynchronization therapy	STRONG	HIGH
Given the limited number of available studies, three-dimensional echocardiography with UEAs is not currently recommended	STRONG	LOW
In patients undergoing resting transthoracic echocardiography alone, there is insufficient evidence to support the use of UEAs when an adequate acoustic window is present, and there are no other reasons for its administration.	STRONG	MODERATE

UEAs: ultrasound-enhancing agents.

5. Clinical Applications in Cardiovascular Stress Studies

5.1. Ultrasound Enhancing Agents during Stress Echocardiography for Cavity Opacification and Assessment of Myocardial Perfusion

Although pharmacological or physical exercise stress echocardiography has shown high sensitivity and specificity in detecting myocardial ischemia, multiple factors can lead to a decrease in the definition of endocardial borders, mainly during peak stress, which may contribute to inadequate image quality (insufficient for diagnostic purposes) in up to 30% of patients.

In this regard, left ventricular opacification using ultrasound-enhancing agents and RTMPE technology has proven essential in obtaining more precise and efficient results in stress echocardiography,[61] improving its diagnostic quality and accuracy[62-64] and its prognostic value.[65]

Prospective multicenter and single-center studies have demonstrated the effectiveness of UEAs in detecting changes in regional wall motion ([65-67]). In addition to enhancing the endocardial border, RTMPE technology allows for subendocardial delineation and analysis of wall thickening and myocardial perfusion, facilitating the detection of subendocardial ischemia.[68] Integration of UEA assessment of LV opacification and myocardial perfusion in the routine evaluation of patients with left bundle branch block during stress echocardiography also demonstrated improved detection of coronary artery disease, in addition to being an independent predictor of cardiovascular mortality.[69]

In a study with 68 patients undergoing dobutamine stress echocardiography using harmonic imaging combined with PESDA for the purpose of endocardial border delineation, 23 patients (34%) who had an inadequate acoustic window without the use of UEAs showed better definition of cardiac borders when using UEAs. Of the 2176 myocardial segments analyzed (1088 at rest and 1088 at peak dobutamine infusion), complete ventricular cavity filling was obtained in all patients. Adequate delineation of endocardial borders increased from 81% of analyzed segments without UEAs to 95% with their use ($p < 0.05$).[70]

The absence of ionizing radiation, coupled with improved endocardial border delineation and additional study of myocardial perfusion at rest and under stress, have made RTMPE technology the standard for performing stress echocardiography studies. Numerous isolated or comparative studies with other methods have demonstrated its superiority or equivalence in terms of diagnostic accuracy and prognostic risk stratification (Figure 18).[70-79]

5.2. Qualitative and Quantitative Analysis of Myocardial Perfusion

The analysis of myocardial perfusion during stress echocardiography, enhanced by the use of UEAs, increases diagnostic sensitivity for the detection of coronary artery disease compared to isolated evaluation of myocardial contractility, since perfusion changes precede regional wall motion abnormalities (RWMAs) in the ischemic cascade.[25,80,81]

This approach allows for the simultaneous, real-time evaluation of myocardial perfusion and subtle, transient RWMAs, hence also increasing the diagnostic accuracy of dipyridamole and adenosine

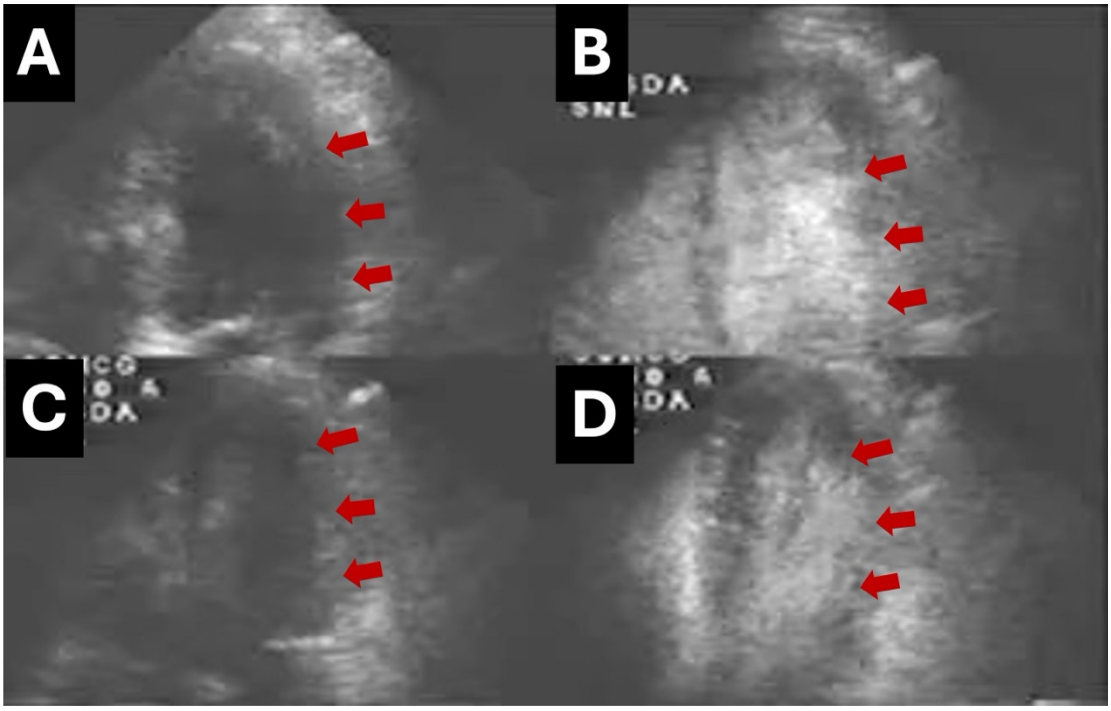


Figure 18 – *Example of endocardial border delineation during dobutamine stress echocardiography with end-systolic images. (A and B): At rest, from left to right without (A) and with (B) ultrasound-enhancing agents (UEAs). (C and D): During peak stress, without (C) and with (D) UEAs. Note the improvement in border delineation in the images with UEAs (arrows).*

stress echocardiography, making them comparable to that of exercise or dobutamine stress echocardiography, in addition to increasing medium- and long-term prognostic value[25, 80] and cost-effectiveness when compared to nuclear studies.[82-85] Economic analyses demonstrate that a strategy based on the use of UEAs reduces rework and the need for additional methods, with cost reductions of around 28% compared to myocardial scintigraphy in outpatient populations (Figure 19).[86]

One study[87] demonstrated that qualitative analysis of myocardial perfusion by echocardiography with UEAs has sensitivity and specificity comparable to scintigraphy, consolidating the method as a promising tool for rapid and safe assessment of myocardial perfusion.

Although visual or qualitative analysis of myocardial perfusion provides additional information on wall motion, the use of quantitative parameters, especially microvascular blood flow velocity reserve (β reserve), is the most objective method, as it is independent of ultrasound field inhomogeneities for the evaluation of patients with coronary artery disease. A prospective head-to-head comparative study in which patients (n = 168) with suspected CAD and preserved left ventricular function underwent both dobutamine and adenosine stress echocardiography showed that quantitative variables, especially β reserve, were independent predictors of cardiac events (cardiac death, myocardial infarction, and unstable angina) in both groups (Figures 19 and 20).[24] An example of a patient who had low myocardial flow velocity reserve (β reserve) and experienced a cardiac event is depicted in Figure 19.

Studies that used quantitative or semi-quantitative analysis in stress echocardiography with UEAs for the detection of coronary artery disease (CAD) demonstrated good diagnostic performance compared to coronary angiography or other non-invasive stress imaging methods,[23,24,75] with one meta-analysis demonstrating sensitivity and specificity above 80% and significant superiority in predicting major cardiovascular events.[78,79,88,89]

For patients with heart failure with reduced ejection fraction (HFrEF), quantitative assessment has proven capable of differentiating ischemic from non-ischemic etiology and predicting mortality. It has also been used to evaluate microvascular dysfunction in non-ischemic hypertensive cardiomyopathy, stress cardiomyopathy, and in patients with angina with non-obstructive coronary arteries (ANOCA).[90,91]

Multicenter studies have been conducted comparing RTMPE with single-photon emission computed tomography (SPECT) using vasodilators for the detection of coronary artery disease. The first study showed equivalent sensitivity and specificity. However, in another blinded, core-lab adjudicated study with a larger number of patients, using

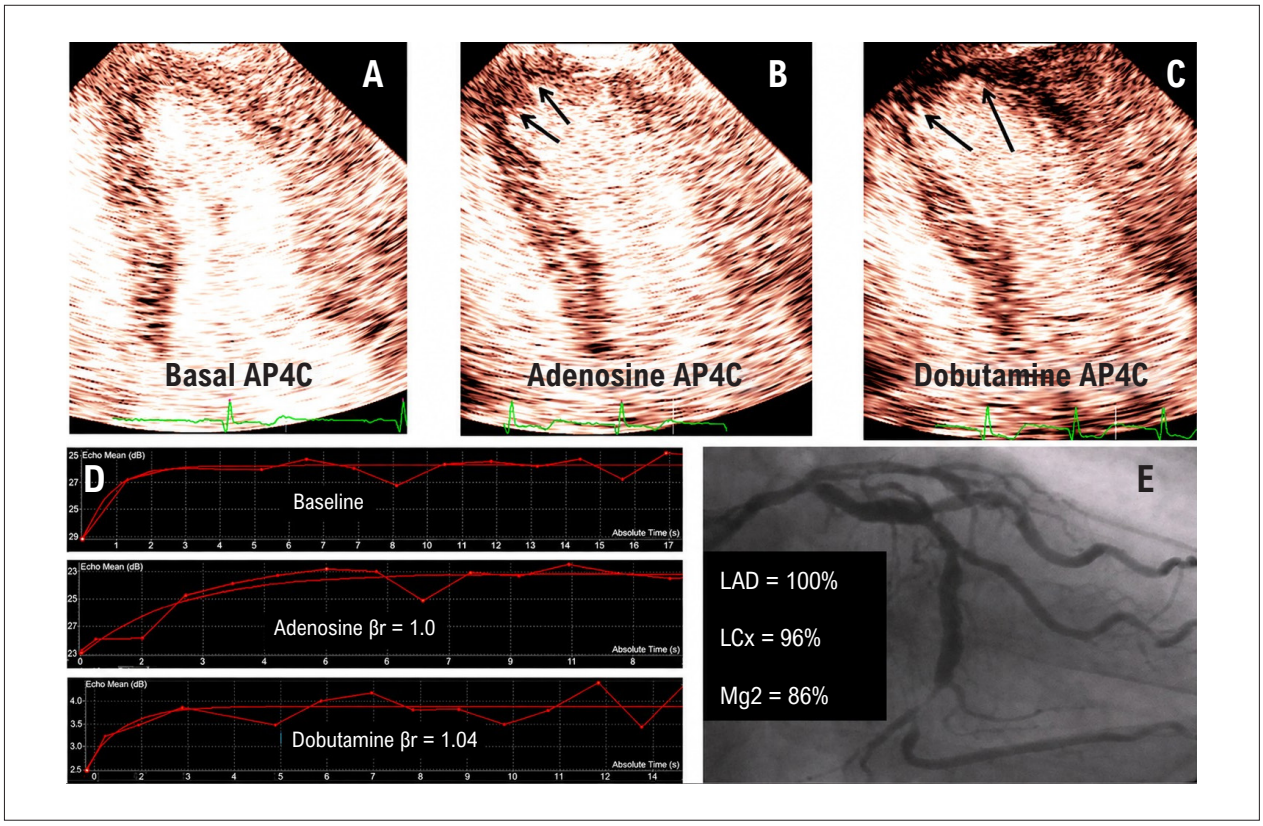


Figure 19 – *Apical 4-chamber view of two pharmacological stress tests in the same patient, combined with microbubble enhancing agents to assess wall motion and perfusion. In (A), normal perfusion is noted at rest. In (B), a transmural perfusion defect in a single apical segment is seen during adenosine stress, and in (C), during dobutamine stress, an extensive transmural apical perfusion defect is seen in at least two segments, with associated wall motion abnormality in the same coronary territory (akinesia). In (D), acoustic intensity/time curves demonstrate a very depressed coronary flow velocity reserve (β reserve) during both stress modalities. In (E), coronary angiography in the left anterior oblique view demonstrating severe obstruction in the left anterior descending and circumflex arteries. AP4C: apical four-chamber view; LAD: left anterior descending coronary artery; LCx: left circumflex coronary artery; Mg2: second marginal branch.*

coronary angiography as the gold standard, the sensitivity and prognostic power of stress echocardiography with UEAs demonstrated greater predictive value for cardiac events alone and when compared to SPECT.[71,92-95]

5.3. Myocardial Viability Assessment

Hibernating myocardium corresponds to a state of chronic contractile dysfunction secondary to sustained myocardial hypoperfusion, but potentially reversible after restoration of regional blood supply.[96] From a clinical standpoint, the central question is whether this dysfunctional myocardium remains viable – that is, whether it has the potential for functional recovery after revascularization.

The assessment of myocardial viability can be performed using non-invasive imaging methods that investigate perfusion, metabolism, and contractile reserve. Among these, the following stand out: myocardial perfusion scintigraphy by single-photon emission computed tomography (SPECT), which estimates residual myocardial perfusion; positron emission tomography (PET), especially with glucose metabolic markers, capable of identifying hypoperfused but metabolically active myocardium; cardiovascular magnetic resonance (CMR) imaging with late gadolinium enhancement, which delineates the extent of necrosis and fibrosis and, therefore, the likelihood of functional recovery; and low-dose dobutamine stress echocardiography (DSE), which assesses segmental contractile reserve as an indirect marker of viability. Recent guidelines for chronic coronary artery disease and cardiovascular imaging recognize these modalities as established approaches for myocardial viability identification in supporting revascularization decisions.[97]

One study[98] reviewed different methods for detecting myocardial viability, focusing on their ability to predict regional and global functional recovery, symptom improvement, exercise tolerance, and prognostic outcomes. Regarding regional functional recovery, 18-fluorodeoxyglucose PET (FDG-PET) showed the highest sensitivity, followed by myocardial scintigraphy with thallium and technetium. Overall, nuclear techniques (FDG-PET and SPECT) showed greater sensitivity in identifying viable myocardium compared to dobutamine stress echocardiography (DSE), which

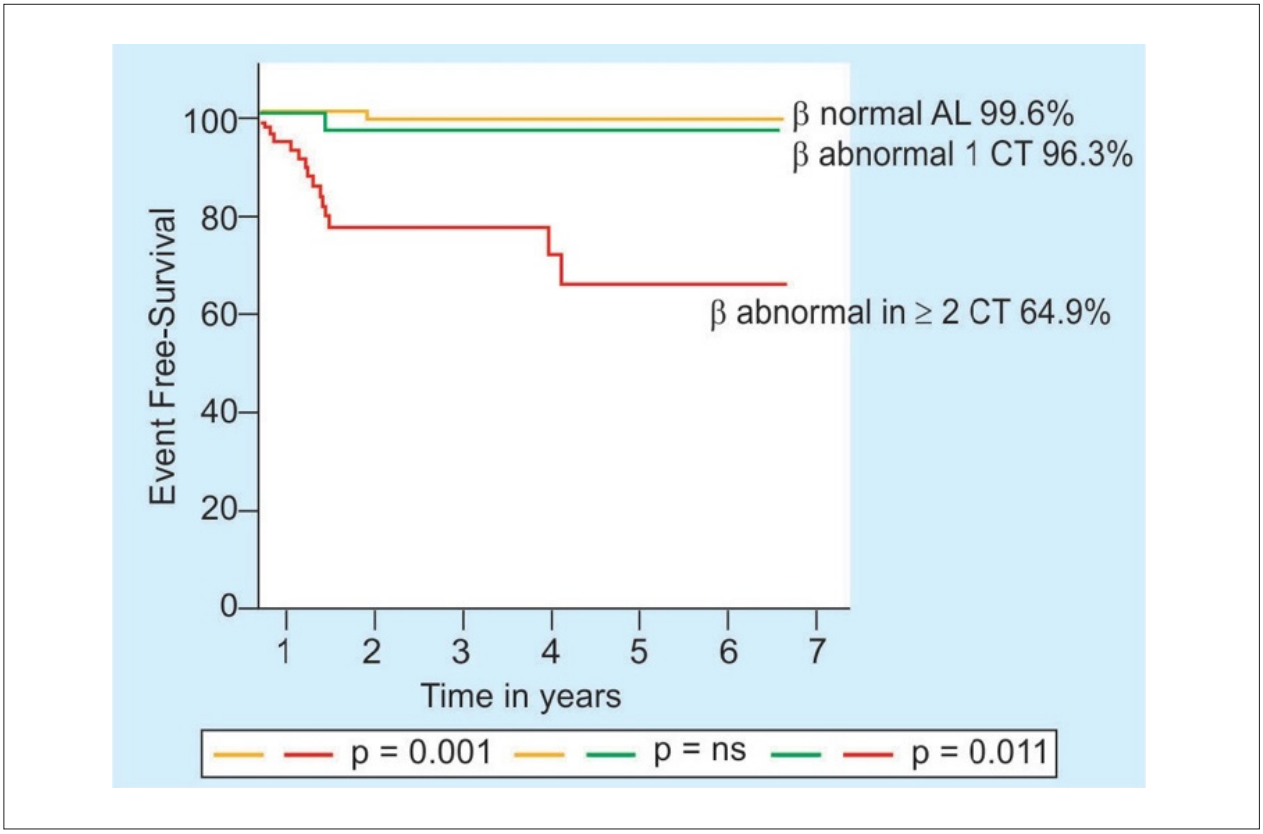


Figure 20 – *Kaplan-Meier curves of patients with normal β-reserve, abnormal β-reserve in only one coronary artery territory, and abnormal β-reserve in two or more territories. AL: all territories; CT: coronary territories.*

showed greater specificity. It is important to note that this review did not systematically evaluate RTMPE using UEAs, which represents a distinct approach by directly estimating microvascular perfusion.

Several clinical series describe RTMPE as a useful tool for assessing myocardial viability after ST-segment elevation myocardial infarction (STEMI) and for characterizing the no-reflow phenomenon. In these scenarios, RTMPE using UEAs demonstrated high sensitivity (62–90%) for detecting viable myocardium, although with lower specificity (18–67%) for left ventricular functional recovery, resulting in a high negative predictive value.[99-101]

Sbano et al.[102], in a study of patients in the first week after myocardial infarction reperfused by thrombolysis, used DSE with UEAs and intermittent harmonic imaging to assess myocardial perfusion and predict ventricular functional recovery. DSE showed high accuracy (90%) and an excellent negative predictive value, precisely identifying segments with contractile reserve that would show late improvement and those in which absence of microvascular perfusion practically ruled out functional recovery.

More recent prospective studies have added that RTMPE combined with low-dose DSE, performed early (approximately 72 hours) after percutaneous coronary intervention in patients with STEMI, identifies viable segments with high accuracy and prognostic value when compared to SPECT-MIBI.[103]

Another study[104] evaluated patients with chronic ischemic heart disease and ventricular dysfunction who underwent coronary artery bypass grafting (CABG), demonstrating that RTMPE was capable of predicting regional functional recovery.

The estimation of capillary blood volume by RTMPE correlates directly with the density and area of the microvasculature and inversely to the collagen content (fibrosis), which determines long-term prognosis.[100,101, 105,106]

One emerging application for assessment of viability using RTMPE is in heart failure with preserved ejection fraction (HFpEF). In these patients, quantitative analysis of myocardial blood flow has shown to be independently associated with adverse clinical events and, when combined with global longitudinal strain (GLS), has increased the capacity for risk stratification in HFpEF patients.[107]

Another emerging application is the acquisition of ultrafast (high frame-rate/ultrafast frame-rate) RTMPE imaging, as recently demonstrated in humans, allowing for the evaluation of myocardial perfusion in the context of coronary artery disease (CAD) with a higher signal-to-noise ratio and better temporal sampling compared to conventional acquisition. This temporal gain potentially improves the estimation of quantitative perfusion parameters.[108] Although still in the translational phase, this technology has the potential to refine the segmental quantification of perfusion and, therefore, allow inference of myocardial viability with increased precision.[109]

5.4. Summary of Recommendations for the use of Ultrasound-Enhancing Agents during Stress Echocardiography

Recommendations	Strength of recommendation	Certainty of evidence
During exercise or pharmacologic stress echocardiography, UEAs should be used to improve endocardial border delineation when two or more contiguous left ventricular segments are not adequately visualized on baseline apical views	STRONG	HIGH
Myocardial perfusion analysis using real-time myocardial perfusion echocardiography with UEAs should be considered during stress echocardiography to maximize diagnostic accuracy and enhance prognostic assessment	STRONG	MODERATE

UEAs: ultrasound-enhancing agents.

6. Use Of Ultrasound-Enhancing Agents in Examinations of the Aorta, Carotid and Vertebral Arteries

UEAs are used to enhance certain structures of similar densities and to assess tissue perfusion. Therefore, they have multiple applications in vascular scans, ranging from improving the identification of lesions in trunk vessels to studying microcirculation within a single plaque. Many of these applications are not within the scope of this document, which is dedicated to vessels of greater interest in cardiology, especially the aorta and carotid arteries.

In vascular diagnostic ultrasound, UEAs protocols and agents are the same as those used for cardiac analysis. In Brazil, SonoVue® has been approved by ANVISA for use in Doppler optimization and in evaluation of the micro- and macrovasculature, being the only ultrasound enhancing agent approved for clinical use in the country to date.

The applications of UEAs in the vascular examinations currently most requested in clinical practice are the study of carotid plaque morphology, carotid neovascularization, and the detection of endoleaks after endovascular treatment of the aorta.

Detection of markers of vulnerability in a carotid atheromatous plaque is essential for evaluation in patients with stroke or at increased risk of stroke[110] by adequately characterizing the surface of the plaque, detecting ulcers and the presence of neovascularization (Figure 21).

Several studies have compared UEAs with non-enhanced ultrasound, demonstrating that UEA-enhanced imaging has a high sensitivity for the diagnosis of plaque ulceration (88–94%) when the reference standard is computed tomography.[111]

Processes such as intraplaque hemorrhage (IPH), currently considered the main marker of vulnerability, are preceded by the formation of new vessels (neovascularization), which promote plaque instability and burst, flooding the lipid-rich core and leading to rapid stenosis progression or plaque rupture, with exposure of subendothelial material and consequent vascular thrombosis. The presence of a plaque with a hypoechoic zone combined with signs of neovascularization can be considered equivalent to IPH and is associated with a higher incidence of cerebrovascular events.

The use of UEAs has proven accurate in detecting and classifying endoleaks associated with endovascular aortic repair. This is due to the possibility of detecting the direction of flow, confirming late, positional, and low-flow endoleaks, and also assisting in the differential diagnosis of endotension, which is defined as a persistently increased state of pressure within the aneurysmal sac in the absence of leaks detectable by usual methods (Figure 22).[112,113]

For this purpose, we generally start with a 1 mL bolus of SonoVue® solution to characterize initial filling of the aorta, followed by detection of any leaks, if present. Good UEA saturation is expected to last up to 5 minutes. After this time, additional boluses (0.5 mL each) are administered at the physician's discretion.

7. Use in Pediatric Cardiology

The use of UEAs in pediatric echocardiography has been expanding progressively, driven by the need to overcome the specific technical challenges of this population, such as in the case of children with congenital heart disease who have limited "acoustic windows" resulting from previous cardiac surgeries, alterations in thoracic geometry, or, more recently, the increased prevalence of childhood obesity. Adolescents and adults with congenital heart disease may also present with these same conditions.

In this context, both UEAs for contrast enhancement of the pulmonary circulation and those that allow whole-circulation enhancement represent valuable tools, since they provide better image quality without exposure to ionizing radiation. This improvement in image quality not only increases diagnostic confidence but can also expedite and modify clinical management, as well as reduce the need for more invasive, complex, and costly diagnostic modalities.[114,115]

This section of the guideline aims to synthesize the most recent global evidence on the efficacy and safety of UEAs in pediatric echocardiography, addressing their clinical indications, safety profile, contraindications, and specific challenges.

7.1. Clinical Applications of Whole-Circulation Ultrasound-Enhancing Agents in Pediatric Echocardiography

The scientific literature on the safe and effective use of UEAs for pediatric echocardiography and echocardiographic imaging of congenital heart conditions is growing, consolidating and reinforcing the role of these agents in modern cardiology practice. The main indications according to the international literature include:

- Suboptimal "acoustic windows": UEAs can be used in children and adolescents in whom conventional echocardiography is inadequate or insufficient for the precise

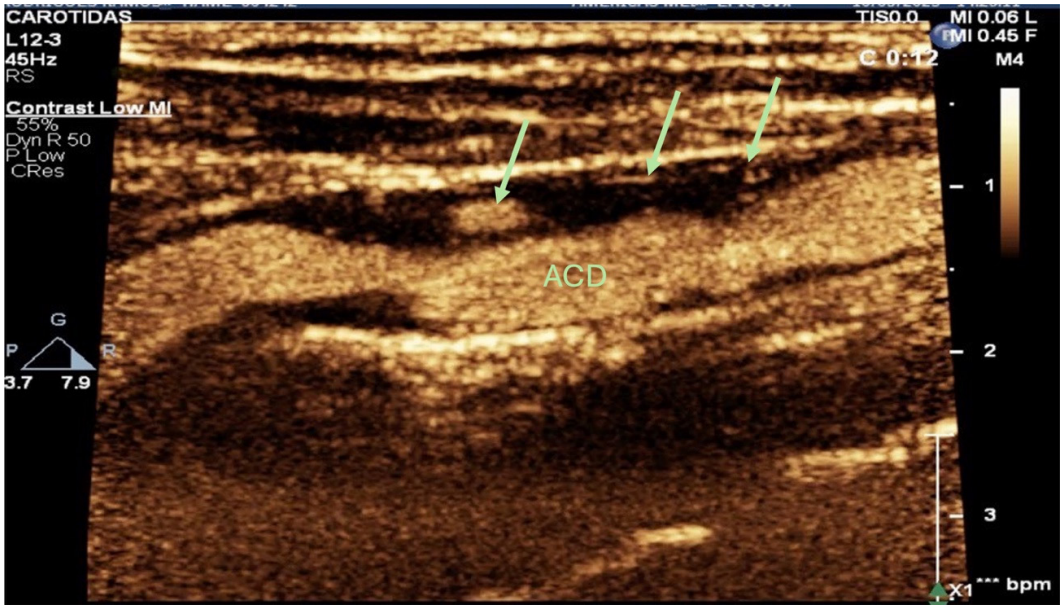


Figure 21 – *Intraplaque neovascularization in carotid stenosis, visualized with UEA enhancement (green arrows). RCA: right carotid artery.*

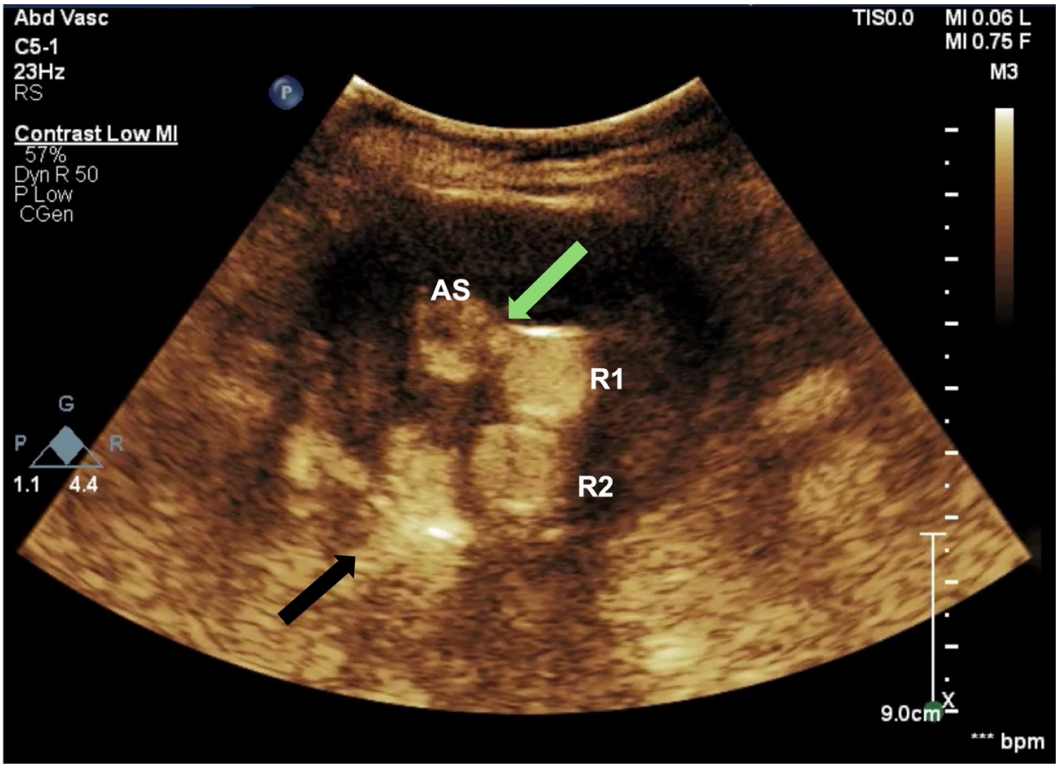


Figure 22 – *Cross section at the level of the distal abdominal aorta showing 2 branches of the endograft (R1/R2) within the aneurysmal sac. Two sites of leakage are evident: a type III endoleak (originating from the graft; green arrow) and a type II endoleak (originating from a lumbar branch; black arrow). AS: aneurysmal sac; R1: branch 1; R2: branch 2.*

definition of endocardial borders, especially after surgical interventions or in cases of chest wall deformities.

• Evaluation of congenital heart disease: UEAs are an option when there is a need to significantly improve the visualization of complex cardiac structures and functional assessment, providing increased diagnostic accuracy and reducing the need for modalities that expose the patient to ionizing radiation (such as computed tomography) or require sedation or general anesthesia (such as magnetic resonance imaging), including postoperatively and at the bedside, when clinical conditions may hinder adequate evaluation by conventional methods.[115]

• Quantification of ventricular function: UEAs increase accuracy in delineating the ventricular endocardial borders, allowing more reliable estimation of volumes and systolic function.[115]

• Estimation of intracardiac pressures: UEAs intensify Doppler signals, such as the tricuspid regurgitation jet, facilitating a more accurate estimation of right ventricular systolic pressure.[116]

• Myocardial ischemia assessment: indicated for patients that have undergone cardiac surgery involving manipulation of the coronary arteries, such as correction of transposition of the great arteries with the Jatene's technique; in the correction of anomalous origins or pathways; or in conditions potentially impairing coronary flow reserve, such as Kawasaki disease or coronary fistulas.[115]

7.2. Safety and Adverse Effects of Ultrasound-Enhancing Agents in Pediatrics

Although ANVISA (the Brazilian Health Regulatory Agency) does not yet recognize the safety of using UEAs in the pediatric population, the international literature has consistently demonstrated their safety and efficacy in this population, especially in the presence of congenital heart disease or suboptimal "acoustic windows." Guidelines issued by international societies, such as the American Society of Echocardiography, recognize the clinical utility of these agents in children and adolescents, emphasizing the ability of UEAs to improve the definition of endocardial borders and ventricular functional assessment, with a risk profile that is considered low.[115,117]

The FDA's 2019 approval of SonoVue® for echocardiography in pediatric patients was based on a body of evidence from clinical studies and retrospective analyses, supported by entities such as the International Contrast Ultrasound Society and the Society for Pediatric Radiology. Among the most relevant studies is a retrospective multicenter analysis conducted by Kutty et al., which evaluated 113 pediatric patients aged 5 to 21 years. The results demonstrated significant improvement in left ventricular opacification, myocardial perfusion assessment, and detection of regional wall motion abnormalities, without any serious adverse events. The few reported symptoms were mild, self-limiting, and did not require therapeutic intervention.[115]

Likewise, other authors[118] prospectively reported that UEA use in children and adolescents aged 9 to 18 years was associated with substantial improvement in the visualization of left ventricular wall segments, as well as a reduction in examinations classified as suboptimal, without recording significant adverse effects.

Taken together, the adverse events reported in the current literature were mostly mild and transient, including symptoms such as chest discomfort, dizziness, headache, and fatigue, all of which were resolved spontaneously. No relevant hemodynamic changes were identified, even in subgroups traditionally considered to be at higher risk, such as children with pulmonary hypertension or in the postoperative period of cardiac surgery.[115-118] The accumulated clinical experience is more robust in school-aged children and adolescents (aged 5 years and older), while data in infants and preschoolers remain limited.

Allergic reactions are rare, with a prevalence of less than 1%, and are more frequently observed in patients with a history of hypersensitivity to iodinated contrast agents. Even so, their risk does not compromise routine use of UEAs in pediatric clinical practice, provided that the usual safety precautions are followed.[115, 118]

Among the new frontiers for UEAs use in the pediatric population, sonothrombolysis (see below in the specific section) seems to be a very promising technique. This was demonstrated in a case report of a 13-month-old infant with hypoplastic left heart syndrome status post second-stage palliative Glenn surgery (anastomosis of the right superior vena cava to the right pulmonary artery), combined with pulmonary artery banding and stenting of the left pulmonary artery, who developed thrombosis and severe clinical instability. After an unsuccessful first attempt at unblocking by catheter-directed thrombolysis with alteplase, the sonothrombolysis technique was then chosen as adjuvant therapy, performed simultaneously with percutaneous hemodynamic reintervention, which culminated in complete recanalization of the coronary circulation.[119]

• The main contraindications for use of UEAs in pediatric echocardiography are:

• Significant intracardiac right-to-left shunt (only for pulmonary-circulation UEAs).

• History of severe allergic reaction (anaphylaxis) to UEA components: allergic reactions are rare but constitute an absolute contraindication.[120,121]

• Conditions associated with severe clinical instability, such as shock or acute respiratory failure, where the risk of adverse events may be magnified.[9]

• Children under 5 years of age: In this age group, relative contraindications include severe pulmonary hypertension and decompensated heart failure, in which use may be considered on a case-by-case basis with close monitoring.[120,121]

7.3. Recommended Doses of Ultrasound-Enhancing Agents in Pediatrics

According to recommendations from the American Institute of Ultrasound in Medicine, the Society for Pediatric Radiology, and the International Contrast Ultrasound Society, the recommended dose of the SonoVue® enhancing agent for use in pediatric echocardiography is 0.03 mL/kg intravenously, with a maximum dose of 2.4 mL per injection.[117,122] Up to 4.8 mL may be administered for a single scan, as approved by the FDA.[117] The agent should be administered by intravenous bolus injection, followed by a slow flush with saline solution.

7.4. Indications for the use of Pulmonary-Circulation Ultrasound-Enhancing Agents (Agitated Saline Solution) in Pediatric Echocardiography

Agitated saline solution (0.9% NaCl) is composed of microbubbles with an average diameter of approximately 100 μm and no ability to cross the pulmonary capillary bed. One of the main indications for its use is when the presence of small or poorly defined interatrial communications is suspected.[14,123]

In summary, the main indications for the use of agitated saline solution in pediatric echocardiography are:

- Detection and characterization of intracardiac shunts: especially interatrial communications, such as patent foramen ovale and atrial septal defect, when conventional images are insufficient or questionable for diagnostic clarification.
- Identification of intrapulmonary shunts: pulmonary arteriovenous malformations secondary to hepatopulmonary syndrome,[124] as observed in Figure 23.
- Investigation of arteriovenous fistulas in univentricular hearts, as shown in Figure 23 (secondary to failure of the cavopulmonary venous return system).[125]
- Identification of anomalous venous structures, such as persistent left superior vena cava or unroofed coronary sinus.
- Clinical monitoring is mandatory during the atrial septal defect occlusion procedure, and contraindications such as a significant right-to-left shunt must be respected.[126]

According to the American Society of Echocardiography guidelines, agitated saline solution can also be used to confirm pericardial access in echocardiography-guided pericardial effusion puncture and drainage procedures.[127] Figure 24 shows this technique, which contributes to the safety of the procedure by allowing visual verification of the needle location before dilation and catheter insertion.

8. Safety and Cost-Effectiveness

8.1. Safety and Indications of Ultrasound-Enhancing Agents in Adults

After approximately two decades of clinical use, UEAs can be considered extremely safe, provided that recommendations for their proper preparation and administration are followed.[54,81,128-130] Serious adverse events are exceedingly rare, with a prevalence of approximately 1 per 10,000 administrations, with anaphylactoid reactions being the most significant.[54,121,130] Specifically regarding the SonoVue® enhancing agent (sulfur hexafluoride core) used in Brazil, Furtado et al.[131] in their safety study reported a very low incidence of allergic reactions (0.6%) in a population of 1099 patients who received this agent. In general, allergic reactions occur immediately or within 30 minutes of administration, are mild in severity, and are transient. The most common presentation is the development of an erythematous rash and urticaria. If this occurs, special attention should be paid to respiratory signs and symptoms (such as dyspnea, laryngeal stridor, and bronchospasm) and to signs of progression to circulatory collapse (such as dizziness, pallor, and hypotension), as these represent early warning signs of the onset of anaphylactic shock. The patient should be carefully evaluated and observed and appropriate treatment for anaphylaxis (including antihistamines, corticosteroids, and epinephrine) promptly instituted as needed, following established protocols.

In October 2007, the FDA added a black box warning to the package inserts of Definity® and Optison® regarding use in acutely ill patients after analyzing 11 deaths potentially related to use of these agents. This warning was later removed after extensive evidence of safety was demonstrated by subsequent clinical

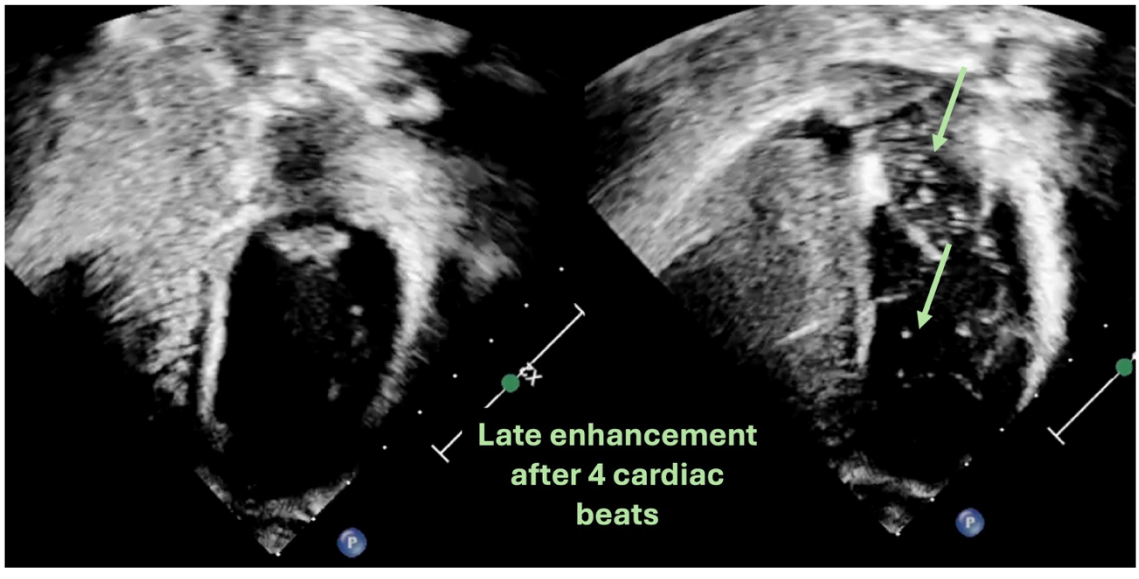


Figure 23 – *Transthoracic echocardiogram after infusion of agitated saline solution via a peripheral IV line in the upper extremity, showing late passage of bubbles to the left heart (green arrows).*

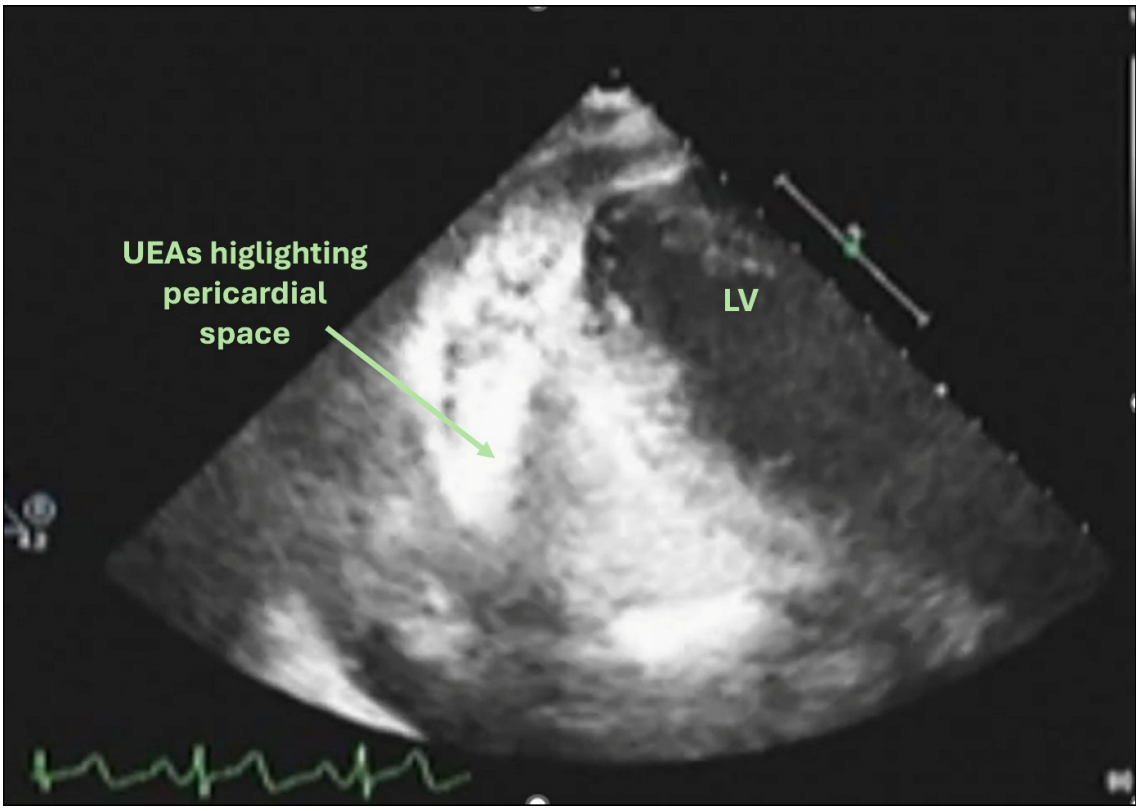


Figure 24 – *Transthoracic echocardiogram showing infusion of agitated saline bubbles during pericardiocentesis (green arrows). LV: left ventricle; UEAs: ultrasound-enhancing agents.*

studies. Advanced lung disease and pulmonary hypertension were initially considered contraindications, but clinical trials ratified the safety of UEAs in these settings,[131-133] as well as in suspected or confirmed significant intracardiac shunt, the latter contraindication having been removed by the FDA in 2016.[120]

Currently, the only absolute contraindication to UEAs is a history of allergic reaction to any component of the specific agent being administered. One noteworthy such example is allergy to polyethylene glycol (PEG), a stabilizing chemical compound present in the excipients of lipid-based UEAs; PEG is also present in various medications (and is itself used as a laxative) and cosmetics.[134]

Supporting this safety profile is the fact that, in clinical scenarios where UEAs are used, often in the most technically challenging examinations and in critically ill patients, numerous studies have shown no increase in morbidity or mortality outcomes. Additionally, it is also valid to argue that UEAs can provide important insights in the critical care setting, such as by facilitating assessment of left ventricular systolic function in a critically ill patient with a significantly limited echocardiographic window or searching for intracavitary thrombus, enabling changes in management with the potential for a significant positive impact on outcome and prognosis.[54,56,135]

8.2. Cost-Effectiveness of Ultrasound-Enhancing Agents

Ultrasound-enhancing agents (UEAs) demonstrate a favorable cost-effectiveness profile when considering the relationship between the cost of the method and its clinical efficiency.

Technically challenging echocardiograms are common, particularly in the emergency department and intensive care unit, due to limited acoustic windows, patient positioning, or restricted access to the chest. In such situations, assessment of left ventricular systolic function may become imprecise or inconclusive, often leading to the need for additional, sometimes more costly imaging modalities, such as cardiac magnetic resonance (CMR).[43,135-138]

In this context, UEAs allow accurate delineation of left ventricular volumes and precise calculation of ejection fraction, frequently eliminating the need for additional imaging. Their use improves echocardiographic accuracy and reproducibility, achieving performance comparable to that of CMR, which is considered the gold standard. This has a direct impact on clinical decision-making and reinforces the favorable cost-effectiveness of UEAs, including in patients with morbid obesity.[138] Furthermore, UEA-enhanced echocardiography is virtually the only diagnostic method capable of confirming myocardial rupture at the bedside in critically ill patients.

In short, UEAs have demonstrated cost-effectiveness across several cardiology scenarios, with particularly strong evidence in critical care and stress echocardiography, where they reduce intra- and interobserver variability.[55,89,129,139,140]

In chest pain units, especially among obese patients, the integration of rest and stress echocardiography using low–MI real-time perfusion imaging (RTMPE) for the assessment of regional wall motion and perfusion represents an effective alternative to more expensive tests, such as coronary computed tomography angiography and myocardial perfusion scintigraphy. This approach is less costly, radiation-free, and highly accurate, reducing suboptimal studies by more than 30%, avoiding the need for additional imaging modalities, and allowing safe patient discharge with annual cardiovascular event rates below 1%.[59,141,142]

9. Emerging Applications

9.1. Sonothrombolysis

Sonothrombolysis is an emerging therapeutic technology that uses high-energy ultrasonic pulses combined with an UEA to dissolve thrombi. Its potential use was initially demonstrated in 2009 in a thrombotic arteriovenous graft canine model. High-mechanical-index pulses were shown to induce inertial cavitation within the graft, promoting thrombi dissolution without the need for fibrinolytic agents.[143] Subsequently, it was demonstrated that sonothrombolysis is also capable of acting on the vascular endothelium and microcirculation, releasing vasodilating substances including nitric oxide.[144] This knowledge served as the foundation for the use of this technique in clinical studies.

Pioneering randomized clinical trials in humans tested the adjunctive use of sonothrombolysis in acute myocardial infarction.[145-150] These studies have demonstrated that the use of ultrasound combined with commercially available UEAs in patients with ST-segment elevation myocardial infarction, randomized to receive or not sonothrombolysis as adjuvant therapy to primary percutaneous coronary intervention (PCI), was associated with a reduction in microvascular obstruction, increased recanalization rates of epicardial vessels, and a decrease in the extent of necrotic tissue. Furthermore, a lower degree of ventricular remodeling and sustained improvement in myocardial function were observed in long-term follow-up.

9.2. Molecular Imaging

This is an ultrasound imaging strategy that aims to assist in the visualization, characterization, and quantification of biological processes at the cellular and molecular levels. It can aid in research studying inflammation, thrombosis, apoptosis, necrosis, vascular remodeling, and angiogenesis.[151-154]

9.3. Gene and Drug Delivery

The use of ultrasound-targeted microbubble destruction (UTMD) for the local delivery of drugs and genes is a promising technique because it is a minimally invasive, reproducible method with a high capacity for transferring bioactive molecules to specific sites.[155]

Although ultrasonic energy alone can facilitate gene delivery through the formation of micropores in cell membranes, the addition of microbubbles reduces the acoustic cavitation threshold and markedly increases the efficiency of this transfer, particularly when genes or nucleic acids are incorporated or coupled directly to the microbubble surface.[156,157]

The mechanisms involve the formation of micropores in cell membranes and active calcium-mediated absorption. Both result from the phenomenon of cavitation, shear forces, the formation of microjets by high-amplitude oscillations, and cell membrane deformation related to high acoustic pressure.[158]

Since the microbubbles are located in the intravascular space, delivery and transfer occur predominantly in the vascular endothelium of the insonated tissue, although they can also occur in the extravascular space.[156-161]

While UTMD has potential advantages over other gene delivery techniques, including its non-invasive nature, efforts are focused on improving transfer efficiency by using newer vectors that prolong delivery or promote chromosome insertion.

9.4. Use in the Context of Interventional Echocardiography

9.4.1. Septal Reduction Therapy for Obstructive Hypertrophic Cardiomyopathy

The use of UEAs has gained increasing relevance in the context of structural heart interventions. One form of obstructive asymmetric septal hypertrophic cardiomyopathy is characterized by dynamic obstruction of the left ventricular outflow tract (LVOT), frequently accompanied by systolic anterior motion (SAM) of the mitral valve and secondary mitral regurgitation.[162]

In symptomatic patients refractory to optimized clinical treatment, with a systolic LVOT gradient ≥ 50 mm Hg at rest or during physiological provocation and myocardial thickness ≥ 15 mm, with predominance of hypertrophy in the basal region of the ventricular septum, septal reduction therapy is indicated. This can be performed by surgical myectomy, alcohol septal ablation (ASA) or radiofrequency ablation.[163,164]

ASA involves the controlled injection of absolute ethanol (96–99%) or other liquid embolic agents into an appropriate septal coronary branch, resulting in localized myocardial infarction in the basal segments of the ventricular septum and consequent reduction of the intracavitary gradient. The success of this procedure depends especially on the precise selection of the target septal artery, since anatomical variations and coronary collaterals can lead to infarction of undesired areas, such as the right ventricular free wall or papillary muscles, in addition to increased risk of atrioventricular block or other hemodynamic complications. Isolated coronary angiography often does not accurately define the territory perfused by each septal artery, nor is it able to detect overlap in regional coronary irrigation. In this context, echocardiography using UEAs is essential

in identifying the myocardial perfusion area of the septal branch to be treated, ensuring greater safety and efficacy of this procedure.[165]

Targeted intracoronary injection of a whole-circulation UEA (~2-μm microbubbles), followed by echocardiographic monitoring by transthoracic and/or transesophageal echocardiography, allows direct visualization of the myocardial region perfused by the tested branch, helping to confirm the absence of flow outside areas of therapeutic interest, such as the papillary muscles, free wall of the right ventricle, and basal segment of the inferior and lateral-inferior walls (Supplementary Videos 7,8 and 9). After positioning the balloon catheter in the septal artery, with proximal occlusion, the UEA is injected at a low dose (< 0.5 mL) and the opacified region is visualized, preferably by transthoracic echocardiography with a low MI (< 0.2). If opacification occurs exclusively in the basal ventricular septum, corresponding to the region of the obstructive gradient, the branch is considered suitable for ethanol injection (Figures 25 and 26). Otherwise, if other regions exhibit opacification, another branch or sub-branch should be sought, or the procedure should be terminated and another form of therapy considered.[166,167] Studies confirm that echocardiographic monitoring of ASA increases the accuracy and safety of the procedure, and is considered good practice in referral centers.[168]

9.5. Percutaneous Closure of the Left Atrial Appendage

In percutaneous closure of the left atrial appendage (LAA), the use of UEAs during the pre-procedural phase allows for a more precise characterization of appendage

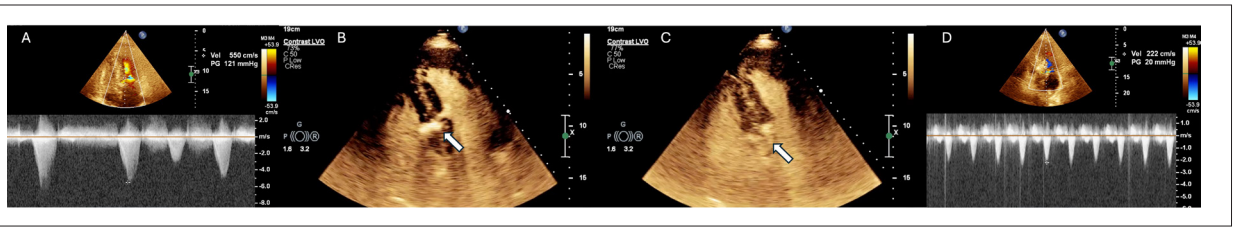


Figure 25 – *Use of ultrasound-enhancing agents (UEAs) during septal reduction therapy. (A): Pre-treatment, with evidence of a peak late systolic left ventricular outflow tract (LVOT) gradient of 121 mm Hg. (B): Use of UEAs after the first attempt at selecting a septal coronary artery, demonstrating perfusion of the mid-segment of the ventricular septum. (C): Use of UEAs after adequate selection of a branch and sub-branch of the septal coronary artery, demonstrating perfusion of the basal segment of the ventricular septum (topography related to the obstructive gradient). (D): Immediately post-treatment, with evidence of a peak late systolic LVOT gradient of 20 mm Hg.*

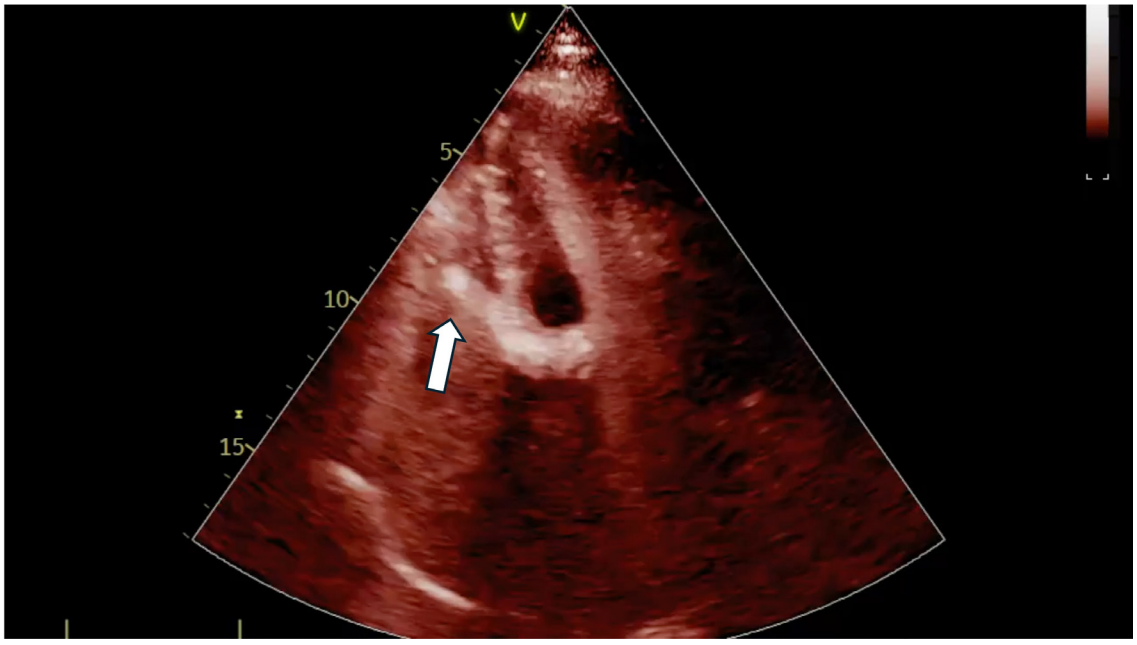


Figure 26 – *Apical 4-chamber view. Use of ultrasound-enhancing agents during alcohol septal ablation with evidence of right ventricular perfusion (moderator band).*

anatomy, including lobe definition, identification of complex trabeculations, and more reliable measurement of ostium diameter and depth.

The evaluation of thrombi, artifacts, or spontaneous contrast in the LAA by transesophageal echocardiography (TEE) is essential in this context. In one study,[169] diagnostic concordance for thrombus detection increased from 72% to 96% when UEAs were used in cases of uncertainty, reducing false positives that would contraindicate the procedure or prolonged anticoagulation. In another analysis, the use of UEAs changed the diagnosis of thrombi in 17% of patients and the management strategy in 13%, highlighting the direct clinical impact of this tool.[170]

During the procedure, UEAs can assist in confirming the location of the closure device before deployment, improving delimitation of the appendage and landing zone borders. In cases of suboptimal visualization during TEE, UEAs contribute to more accurate decision-making regarding device repositioning or recapture.

Although routine use still varies across centers, the literature demonstrates that incorporating UEAs during percutaneous LAA closure can reduce uncertainties, optimize decision-making, decrease procedure duration and, therefore, ionizing radiation exposure for both the patient and the medical team, as well as reduce the need for iodinated contrast administration.[171]

10. Regulatory and Operational Aspects Related to the Incorporation of Ultrasound-Enhancing Agents into Routine Practice in Brazil

In Brazil, the broad and systematic adoption of UEAs depends on harmonious integration among health regulation, represented by the National Health Surveillance Agency (ANVISA); coverage and reimbursement standards approved by the National Supplementary Health Agency (ANS) through its Brazilian Hierarchical Classification of Medical Procedures (CBHPM); and formal health technology assessment processes conducted by the National Commission for the Incorporation of Technologies into the Unified Health System (CONITEC). Incorporating UEAs into routine practice requires technical infrastructure, multiprofessional training, and standardized workflows that ensure quality, safety, and economic feasibility.

SonoVue® is, as of the date of publication of this document, the only whole-circulation microbubble agent approved in Brazil, having been granted marketing authorization by ANVISA in 2013. Approval follows rigorous regulatory guidelines and involves detailed evaluation of safety, efficacy, and quality, as well as pharmacovigilance. Approved indications currently include echocardiography for left ventricular enhancement, vascular studies, and investigation of perfusion in different organs, including the heart.

UEA-enhanced echocardiography has been included in the ANS procedure list since 2014. This ensures mandatory coverage by health insurance providers for recognized indications. The CBHPM defines specific codes and provides additional compensation for procedures involving UEAs; nevertheless, contractual variations between insurance companies and healthcare providers may occur.

The incorporation of UEAs into the Brazilian Unified Health System (SUS) depends on its evaluation by CONITEC, which requires a systematic review of the evidence, a cost-effectiveness study, and an analysis of budgetary impact. Due to their excellent cost-benefit ratio, many centers advocate for the progressive incorporation of UEAs, especially considering the possibility of replacing or reducing more expensive diagnostic modalities such as computed tomography and magnetic resonance imaging, potentially improving the cost-effectiveness of diagnosis and reducing patients' exposure to ionizing radiation and iodinated contrast agents, an advantage that is especially relevant in the pediatric population and in young adults.

UEAs have an excellent safety profile. Basic monitoring should be performed as appropriate for the patient's clinical condition. Adverse events are rare but should be reported to ANVISA in accordance with pharmacovigilance regulations.[172]

Scheduling systems should proactively identify examinations with a higher likelihood of benefiting from UEAs, such as patients with unfavorable "acoustic windows," need for precise quantification of systolic function, or evaluation of masses or thrombi. The use of specific CBHPM codes and adequate documentation of clinical indications facilitate auditing and prevent denials. Clear communication with health insurance companies and the presentation of institutional results should increase acceptance and reduce administrative barriers.

Monitoring rates of inconclusive scans, repeat scans, need for additional diagnostic studies, and average room turnaround time is advised, as these indicators help in evaluating effectiveness and justify institutional investment.

Key challenges include a lack of technical knowledge among auditors and managers, a mistaken perception that UEAs are "experimental," administrative resistance, and a lack of structured internal protocols. Solutions include involvement of scientific societies, development of internal good-practice manuals, holding clinical sessions to disseminate knowledge, transparency in quality indicators, and maintaining a continuous dialogue with health insurance companies and institutional managers.

The implementation of UEAs in Brazilian echocardiography laboratories represents a significant advancement aligned with international best practices. This technology is safe, cost-effective, and widely supported by scientific evidence. Its systematic use improves diagnostic accuracy, reduces variability, and increases the overall efficiency of health services.[109,128]

The consolidation of UEA use in clinical practice depends on planning, standardization, training, and integration among clinical teams, institutional management, and regulatory systems. National and international evidence and experience support the definitive consolidation of this technology as an essential component of modern echocardiography and ultrasound services.

References

1. Porter TR, Mulvagh SL, Abdelmoneim SS, Becher H, Belcik JT, Bierig M, et al. Clinical Applications of Ultrasonic Enhancing Agents in Echocardiography: 2018 American Society of Echocardiography Guidelines Update. J Am Soc Echocardiogr. 2018;31(3):241-74. doi: 10.1016/j.echo.2017.11.013.
2. de Jong N, Ten Cate FJ, Lancée CT, Roelandt JR, Bom N. Principles and Recent Developments in Ultrasound Contrast Agents. Ultrasonics. 1991;29(4):324-30. doi: 10.1016/0041-624x(91)90030-c.
3. Burns PN. Harmonic Imaging with Ultrasound Contrast Agents. Clin Radiol. 1996;51(Suppl 1):50-5.
4. Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, Kaul S. Quantification of Myocardial Blood Flow with Ultrasound-Induced Destruction of Microbubbles Administered as a Constant Venous Infusion. Circulation. 1998;97(5):473-83. doi: 10.1161/01.cir.97.5.473.
5. Gramiak R, Shah PM, Kramer DH. Ultrasound Cardiography: Contrast Studies in Anatomy and Function. Radiology. 1969;92(5):939-48. doi: 10.1148/92.5.939.
6. Feinstein SB, Ten Cate FJ, Zwehl W, Ong K, Maurer G, Tei C, et al. Two-Dimensional Contrast Echocardiography. I. In Vitro Development and Quantitative Analysis of Echo Contrast Agents. J Am Coll Cardiol. 1984;3(1):14-20. doi: 10.1016/s0735-1097(84)80424-6.
7. Porter TR, Xie F, Kricsfeld D, Armbruster RW. Improved Myocardial Contrast with Second Harmonic Transient Ultrasound Response Imaging in Humans using Intravenous Perfluorocarbon-Exposed Sonicated Dextrose Albumin. J Am Coll Cardiol. 1996;27(6):1497-501. doi: 10.1016/0735-1097(96)00017-4.
8. Global Burden of Cardiovascular Diseases and Risks 2023 Collaborators. Global, Regional, and National Burden of Cardiovascular Diseases and Risk Factors in 204 Countries and Territories, 1990-2023. J Am Coll Cardiol. 2025;86(22):2167-243. doi: 10.1016/j.jacc.2025.08.015.
9. Saric M, Armour AC, Arnaout MS, Chaudhry FA, Grimm RA, Kronzon I, et al. Guidelines for the Use of Echocardiography in the Evaluation of a Cardiac Source of Embolism. J Am Soc Echocardiogr. 2016;29(1):1-42. doi: 10.1016/j.echo.2015.09.011.
10. Xu K, Tian X, Hao M, Li Y, Zhang J, Wong RHL, et al. Diagnostic Value of Contrast-Enhanced Ultrasonography for Patent Foramen Ovale Detection. J Thorac Dis. 2024;16(5):3282-90. doi: 10.21037/jtd-24-330.
11. Porter TR, Abdelmoneim S, Belcik JT, McCulloch ML, Mulvagh SL, Olson JJ, et al. Guidelines for the Cardiac Sonographer in the Performance of Contrast Echocardiography: A Focused Update from the American Society of Echocardiography. J Am Soc Echocardiogr. 2014;27(8):797-810. doi: 10.1016/j.echo.2014.05.011.
12. Freeman JA, Woods TD. Use of Saline Contrast Echo Timing to Distinguish Intracardiac and Extracardiac Shunts: Failure of the 3- to 5-beat Rule. Echocardiography. 2008;25(10):1127-30. doi: 10.1111/j.1540-8175.2008.00741.x.
13. Attaran RR, Ata I, Kudithipudi V, Foster L, Sorrell VL. Protocol for Optimal Detection and Exclusion of a Patent Foramen Ovale using Transthoracic Echocardiography with Agitated Saline Microbubbles. Echocardiography. 2006;23(7):616-22. doi: 10.1111/j.1540-8175.2006.00272.x.
14. Bernard S, Churchill TW, Namasivayam M, Bertrand PB. Agitated Saline Contrast Echocardiography in the Identification of Intra- and Extracardiac Shunts: Connecting the Dots. J Am Soc Echocardiogr. 2020:S0894-7317(20)30615-5. doi: 10.1016/j.echo.2020.09.013.
15. Rasalingam R, Novak E, Rifkin RD. Improved Differential Diagnosis of Intracardiac and Extracardiac Shunts using Acoustic Intensity Mapping of Saline Contrast Studies. Eur Heart J Cardiovasc Imaging. 2020;21(3):307-317. doi: 10.1093/ehjci/jez129.
16. Platts DG, Vaishnav M, Burstow DJ, Craig CH, Chan J, Sedgwick JL, et al. Contrast Microsphere Enhancement of the Tricuspid Regurgitant Spectral Doppler Signal - Is it Still Necessary with Contemporary Scanners? Int J Cardiol Heart Vasc. 2017;17:1-10. doi: 10.1016/j.ijcha.2017.08.002.
17. Appadurai V, Kinno M, Minga I, Slostad B, Cascino GJ, Nayak T, et al. The Value of Ultrasound Enhancing Agents in the Echocardiographic Acquisition of Pulmonary Artery Systolic Pressure: An Invasive to Non-Invasive Correlation Study. Int J Cardiovasc Imaging. 2024;40(4):821-30. doi: 10.1007/s10554-024-03051-9.
18. Tan HC, Fung KC, Kritharides L. Agitated Colloid is Superior to Saline and Equivalent to Levovist in Enhancing Tricuspid Regurgitation Doppler Envelope and in the Opacification of Right Heart Chambers: A Quantitative, Qualitative, and Cost-Effectiveness Study. J Am Soc Echocardiogr. 2002;15(4):309-15. doi: 10.1067/mje.2002.116717.
19. Porter TR, Xie F, Kilzer K. Intravenous Perfluoropropane-Exposed Sonicated Dextrose Albumin Produces Myocardial Ultrasound Contrast that Correlates with Coronary Blood Flow. J Am Soc Echocardiogr. 1995;8(5 Pt 1):710-8. doi: 10.1016/s0894-7317(05)80386-4.
20. Lee WS, Lee SC, Kim JM, Shin IS, Jung SS, Kim SJ, et al. Effect of Different Conditioning on Perfluorocarbon Exposed Sonicated Dextrose Albumin Manufacture. J Cardiovasc Ultrasound. 2006;14(4):143-8. doi: 10.4250/jcu.2006.14.4.143.
21. Skyba DM, Jayaweera AR, Goodman NC, Ismail S, Camarano G, Kaul S. Quantification of Myocardial Perfusion with Myocardial Contrast Echocardiography during Left Atrial Injection of Contrast. Implications for venous injection. Circulation. 1994;90(3):1513-21. doi: 10.1161/01.cir.90.3.1513.
22. Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, Kaul S. Basis for Detection of Stenosis using Venous Administration of Microbubbles during Myocardial Contrast Echocardiography: Bolus or Continuous Infusion? J Am Coll Cardiol. 1998;32(1):252-60. doi: 10.1016/s0735-1097(98)00212-5.
23. Xie F, Dodla S, O'Leary E, Porter TR. Detection of Subendocardial Ischemia in the Left Anterior Descending Coronary Artery Territory with Real-Time Myocardial Contrast Echocardiography during Dobutamine Stress Echocardiography. JACC Cardiovasc Imaging. 2008;1(3):271-8. doi: 10.1016/j.jcmg.2008.02.004.
24. Mattoso AA, Kowatsch I, Tsutsui JM, de la Cruz VY, Ribeiro HB, Sbano JC, et al. Prognostic Value of Qualitative and Quantitative Vasodilator Stress Myocardial Perfusion Echocardiography in Patients with Known or Suspected Coronary Artery Disease. J Am Soc Echocardiogr. 2013;26(5):539-47. doi: 10.1016/j.echo.2013.01.016.
25. Pellikka PA, Arruda-Olson A, Chaudhry FA, Chen MH, Marshall JE, Porter TR, et al. Guidelines for Performance, Interpretation, and Application of Stress Echocardiography in Ischemic Heart Disease: From the American Society of Echocardiography. J Am Soc Echocardiogr. 2020;33(1):1-41.e8. doi: 10.1016/j.echo.2019.07.001.
26. Douglas PS, Stebbins A, Foldyna B, Patel MR, Mark DB, Lu MT, et al. Survival after Initial Stress Testing vs Anatomic Testing in Suspected Coronary Artery Disease: Long-Term Follow-Up of the PROMISE Randomized Clinical Trial. JAMA Cardiol. 2025;10(10):1050-4. doi: 10.1001/jamacardio.2025.2882.
27. Harb SC, Marwick TH. Prognostic Value of Stress Imaging after Revascularization: A Systematic Review of Stress Echocardiography and Stress Nuclear Imaging. Am Heart J. 2014;167(1):77-85. doi: 10.1016/j.ahj.2013.07.035.
28. Gaibazzi N, Rigo F, Lorenzoni V, Molinaro S, Bartolomucci F, Reverberi C, et al. Comparative Prediction of Cardiac Events by Wall Motion, Wall Motion Plus Coronary Flow Reserve, or Myocardial Perfusion Analysis: A Multicenter Study of Contrast Stress Echocardiography. JACC Cardiovasc Imaging. 2013;6(1):1-12. doi: 10.1016/j.jcmg.2012.08.009.
29. Marwick TH, Mehta R, Arheart K, Lauer MS. Use of Exercise Echocardiography for Prognostic Evaluation of Patients with Known or Suspected Coronary Artery Disease. J Am Coll Cardiol. 1997;30(1):83-90. doi: 10.1016/s0735-1097(97)00148-4.

30. Lin SS, Lauer MS, Marwick TH. Risk Stratification of Patients with Medically Treated Unstable Angina using Exercise Echocardiography. Am J Cardiol. 1998;82(6):720-4. doi: 10.1016/s0002-9149(98)00462-7.

31. Cortigiani L, Bigi R, Sicari R, Landi P, Bovenzi F, Picano E. Prognostic Value of Pharmacological Stress Echocardiography in Diabetic and Nondiabetic Patients with Known or Suspected Coronary Artery Disease. J Am Coll Cardiol. 2006;47(3):605-10. doi: 10.1016/j.jacc.2005.09.035.

32. Picano E, Lattanzi F, Sicari R, Silvestri O, Polimeno S, Pingitore A, et al. Role of Stress Echocardiography in Risk Stratification Early after an Acute Myocardial Infarction. EPIC (Echo Persantin International Cooperative) and EDIC (Echo Dobutamine International Cooperative) Study Groups. Eur Heart J. 1997;18(Suppl D):D78-85. doi: 10.1093/eurheartj/18.suppl_d.78.

33. Cortigiani L, Picano E, Vigna C, Lattanzi F, Coletta C, Mariotti E, et al. Prognostic Value of Pharmacologic Stress Echocardiography in Patients with Left Bundle Branch Block. Am J Med. 2001;110(5):361-9. doi: 10.1016/s0002-9343(01)00630-1.

34. Sicari R, Picano E, Landi P, Pingitore A, Bigi R, Coletta C, et al. Prognostic Value of Dobutamine-Atropine Stress Echocardiography Early after Acute Myocardial Infarction. Echo Dobutamine International Cooperative (EDIC) Study. J Am Coll Cardiol. 1997;29(2):254-60. doi: 10.1016/s0735-1097(96)00484-6.

35. Cortigiani L, Picano E, Coletta C, Chiarella F, Mathias W, Gandolfo N, et al. Safety, Feasibility, and Prognostic Implications of Pharmacologic Stress Echocardiography in 1482 Patients Evaluated in an Ambulatory Setting. Am Heart J. 2001;141(4):621-9. doi: 10.1067/mhj.2001.113997.

36. Pingitore A, Picano E, Varga A, Gigli G, Cortigiani L, Previtali M, et al. Prognostic Value of Pharmacological Stress Echocardiography in Patients with Known or Suspected Coronary Artery Disease: A Prospective, Large-Scale, Multicenter, Head-to-Head Comparison between Dipyridamole and Dobutamine Test. Echo-Persantine International Cooperative (EPIC) and Echo-Dobutamine International Cooperative (EDIC) Study Groups. J Am Coll Cardiol. 1999;34(6):1769-77. doi: 10.1016/s0735-1097(99)00423-4.

37. Sicari R, Landi P, Picano E, Pirelli S, Chiarandà G, Previtali M, et al. Exercise-Electrocardiography and/or Pharmacological Stress Echocardiography for Non-Invasive Risk Stratification Early after Uncomplicated Myocardial Infarction. A Prospective International Large Scale Multicentre Study. Eur Heart J. 2002;23(13):1030-7. doi: 10.1053/euhj.2001.3072.

38. Shaw LJ, Phillips LM, Leipsic J, Broderick S, Mieres JH, Marwick TH, et al. Comparative Prognosis by Stress ECG and Stress Imaging: Results from the ISCHEMIA Trial. JACC Cardiovasc Imaging. 2025;18(9):943-55. doi: 10.1016/j.jcmg.2025.03.016.

39. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2015;28(1):1-39.e14. doi: 10.1016/j.echo.2014.10.003.

40. Hoffmann R, Barletta G, von Bardeleben S, Vanoverschelde JL, Kasprzak J, Greis C, et al. Analysis of Left Ventricular Volumes and Function: A Multicenter Comparison of Cardiac Magnetic Resonance Imaging, Cine Ventriculography, and Unenhanced and Contrast-Enhanced Two-Dimensional and Three-Dimensional Echocardiography. J Am Soc Echocardiogr. 2014;27(3):292-301. doi: 10.1016/j.echo.2013.12.005.

41. Hoffmann R, von Bardeleben S, Ten Cate F, Borges AC, Kasprzak J, Firschke C, et al. Assessment of Systolic Left Ventricular Function: A Multi-Centre Comparison of Cineventriculography, Cardiac Magnetic Resonance Imaging, Unenhanced and Contrast-Enhanced Echocardiography. Eur Heart J. 2005;26(6):607-16. doi: 10.1093/eurheartj/ehi083.

42. Larsson MK, Silva C, Gunyeli E, Ilami AA, Szummer K, Winter R, et al. The Potential Clinical Value of Contrast-Enhanced Echocardiography beyond Current Recommendations. Cardiovasc Ultrasound. 2016;14:2. doi: 10.1186/s12947-015-0045-0.

43. Hoffmann R, von Bardeleben S, Kasprzak JD, Borges AC, Ten Cate F, Firschke C, et al. Analysis of Regional Left Ventricular Function by Cineventriculography, Cardiac Magnetic Resonance Imaging, and unenhanced and Contrast-Enhanced Echocardiography: A Multicenter Comparison of Methods. J Am Coll Cardiol. 2006;47(1):121-8. doi: 10.1016/j.jacc.2005.10.012.

44. Dwivedi G, Janardhanan R, Hayat SA, Lim TK, Senior R. Improved Prediction of Outcome by Contrast Echocardiography Determined Left Ventricular Remodelling Parameters Compared to Unenhanced Echocardiography in Patients Following Acute Myocardial Infarction. Eur J Echocardiogr. 2009;10(8):933-40. doi: 10.1093/ejechocard/jep099.

45. Uenishi EK, Caldas MA, Tsutsui JM, Abduch MC, Sbano JC, Kalil R Filho, et al. Evaluation of Cardiac Masses by Real-Time Perfusion Imaging Echocardiography. Cardiovasc Ultrasound. 2015;13:23. doi: 10.1186/s12947-015-0018-3.

46. Weinsaft JW, Kim J, Medicherla CB, Ma CL, Codella NC, Kukar N, et al. Echocardiographic Algorithm for Post-Myocardial Infarction LV Thrombus: A Gatekeeper for Thrombus Evaluation by Delayed Enhancement CMR. JACC Cardiovasc Imaging. 2016;9(5):505-15. doi: 10.1016/j.jcmg.2015.06.017.

47. Jung PH, Mueller M, Schuhmann C, Eickhoff M, Schneider P, Seemueller G, et al. Contrast Enhanced Transesophageal Echocardiography in Patients with Atrial Fibrillation Referred to Electrical Cardioversion Improves Atrial Thrombus Detection and May Reduce Associated Thromboembolic Events. Cardiovasc Ultrasound. 2013;11(1):1. doi: 10.1186/1476-7120-11-1.

48. Kirkpatrick JN, Wong T, Bednarz JE, Spencer KT, Sugeng L, Ward RP, et al. Differential Diagnosis of Cardiac Masses using Contrast Echocardiographic Perfusion Imaging. J Am Coll Cardiol. 2004;43(8):1412-9. doi: 10.1016/j.jacc.2003.09.065.

49. Nagueh SF, Bierig SM, Budoff MJ, Desai M, Dilsizian V, Eidem B, et al. American Society of Echocardiography Clinical Recommendations for Multimodality Cardiovascular Imaging of Patients with Hypertrophic Cardiomyopathy: Endorsed by the American Society of Nuclear Cardiology, Society for Cardiovascular Magnetic Resonance, and Society of Cardiovascular Computed Tomography. J Am Soc Echocardiogr. 2011;24(5):473-98. doi: 10.1016/j.echo.2011.03.006.

50. Mulvagh SL, Rakowski H, Vannan MA, Abdelmoneim SS, Becher H, Bierig SM, et al. American Society of Echocardiography Consensus Statement on the Clinical Applications of Ultrasonic Contrast Agents in Echocardiography. J Am Soc Echocardiogr. 2008;21(11):1179-201. doi: 10.1016/j.echo.2008.09.009.

51. Lampropoulos KM, Dounis VG, Aggeli C, Iliopoulos TA, Stefanadis C. Contrast Echocardiography: Contribution to Diagnosis of Left Ventricular Non-Compaction Cardiomyopathy. Hellenic J Cardiol. 2011;52(3):265-72.

52. Senior R, Dwivedi G, Hayat S, Lim TK. Clinical Benefits of Contrast-Enhanced Echocardiography during Rest and Stress Examinations. Eur J Echocardiogr. 2005;6(Suppl 2):S6-13. doi: 10.1016/s1525-2167(05)80723-1.

53. Exuzides A, Main ML, Colby C, Grayburn PA, Feinstein SB, Goldman JH. A Retrospective Comparison of Mortality in Critically ill Hospitalized Patients Undergoing Echocardiography with and without an Ultrasound Contrast Agent. JACC Cardiovasc Imaging. 2010;3(6):578-85. doi: 10.1016/j.jcmg.2010.04.006.

54. Main ML, Hibberd MG, Ryan A, Lowe TJ, Miller P, Bhat G. Acute Mortality in Critically ill Patients Undergoing Echocardiography with or without an Ultrasound Contrast Agent. JACC Cardiovasc Imaging. 2014;7(1):40-8. doi: 10.1016/j.jcmg.2013.08.012.

55. Kurt M, Shaikh KA, Peterson L, Kurrelmeyer KM, Shah G, Nagueh SF, et al. Impact of Contrast Echocardiography on Evaluation of Ventricular Function and Clinical Management in a Large Prospective Cohort. J Am Coll Cardiol. 2009;53(9):802-10. doi: 10.1016/j.jacc.2009.01.005.

56. Main ML, Fu JW, Gundrum J, LaPointe NA, Gillam LD, Mulvagh SL. Impact of Contrast Echocardiography on Outcomes in Critically Ill Patients. Am J Cardiol. 2021;150:117-22. doi: 10.1016/j.amjcard.2021.03.039.

57. Wei K. Utility Contrast Echocardiography in the Emergency Department. JACC Cardiovasc Imaging. 2010;3(2):197-203. doi: 10.1016/j.jcmg.2009.09.019.

58. Rinkevich D, Kaul S, Wang XQ, Tong KL, Belcik T, Kalvaitis S, et al. Regional Left Ventricular Perfusion and Function in Patients Presenting to the Emergency Department with Chest Pain and no ST-Segment Elevation. Eur Heart J. 2005;26(16):1606-11. doi: 10.1093/eurheartj/ehi335.

59. Wyrick JJ, Kalvaitis S, McConnell KJ, Rinkevich D, Kaul S, Wei K. Cost-Efficiency of Myocardial Contrast Echocardiography in Patients Presenting to the Emergency Department with Chest Pain of Suspected Cardiac Origin and a Nondiagnostic Electrocardiogram. Am J Cardiol. 2008;102(6):649-52. doi: 10.1016/j.amjcard.2008.05.008.

60. Pereira JB, Essa M, Ugonabo I, Hur DJ, Crandall I, Vaccarelli M, et al. The Feasibility of Contrast Echocardiography in the Assessment of Right Ventricular Size and Function. Echocardiography. 2019;36(11):1979-88. doi: 10.1111/echo.14504.

61. Plana JC, Mikati IA, Dokainish H, Lakkis N, Abukhalil J, Davis R, et al. A Randomized Cross-Over Study for Evaluation of the Effect of Image Optimization with Contrast on the Diagnostic Accuracy of Dobutamine Echocardiography in Coronary Artery Disease The OPTIMIZE Trial. JACC Cardiovasc Imaging. 2008;1(2):145-52. doi: 10.1016/j.jcmg.2007.10.014.

62. Marwick TH, Nemec JJ, Pashkow FJ, Stewart WJ, Salcedo EE. Accuracy and Limitations of Exercise Echocardiography in a Routine Clinical Setting. J Am Coll Cardiol. 1992;19(1):74-81. doi: 10.1016/0735-1097(92)90054-q.

63. Pellikka PA, Roger VL, Oh JK, Miller FA, Seward JB, Tajik AJ. Stress Echocardiography. Part II. Dobutamine Stress Echocardiography: Techniques, Implementation, Clinical Applications, and Correlations. Mayo Clin Proc. 1995;70(1):16-27. doi: 10.1016/S0025-6196(11)64660-0.

64. Roger VL, Pellikka PA, Oh JK, Miller FA, Seward JB, Tajik AJ. Stress Echocardiography. Part I. Exercise Echocardiography: Techniques, Implementation, Clinical Applications, and Correlations. Mayo Clin Proc. 1995;70(1):5-15. doi: 10.1016/S0025-6196(11)64659-4.

65. Shah BN, Balaji G, Alhajiri A, Ramzy IS, Ahmadvazir S, Senior R. Incremental Diagnostic and Prognostic Value of Contemporary Stress Echocardiography in a Chest Pain Unit: Mortality and Morbidity Outcomes from a Real-World Setting. Circ Cardiovasc Imaging. 2013;6(2):202-9. doi: 10.1161/CIRCIMAGING.112.980797.

66. Dolan MS, Gala SS, Dodla S, Abdelmoneim SS, Xie F, Cloutier D, et al. Safety and Efficacy of Commercially Available Ultrasound Contrast Agents for Rest and Stress Echocardiography a multicenter experience. J Am Coll Cardiol. 2009;53(1):32-8. doi: 10.1016/j.jacc.2008.08.066.

67. Porter TR, Smith LM, Wu J, Thomas D, Haas JT, Mathers DH, et al. Patient Outcome Following 2 different Stress Imaging Approaches: A Prospective Randomized Comparison. J Am Coll Cardiol. 2013;61(24):2446-55. doi: 10.1016/j.jacc.2013.04.019.

68. Thomas D, Xie F, Smith LM, O'Leary E, Smith K, Olson J, et al. Prospective Randomized Comparison of Conventional Stress Echocardiography and Real-Time Perfusion Stress Echocardiography in Detecting Significant Coronary Artery Disease. J Am Soc Echocardiogr. 2012;25(11):1207-14. doi: 10.1016/j.echo.2012.08.016.

69. Vamvakidou A, Karogiannis N, Tzalamouras V, Parsons G, Young G, Gurunathan S, et al. Prognostic Usefulness of Contemporary Stress Echocardiography in Patients with Left Bundle Branch Block and Impact of Contrast Use in Improving Prediction of Outcome. Eur Heart J Cardiovasc Imaging. 2017;18(4):415-21. doi: 10.1093/ehjci/jew211.

70. Mathias W Jr, Arruda AL, Andrade JL, Campos O Filho, Porter TR. Endocardial Border Delineation during Dobutamine Infusion Using Contrast Echocardiography. Echocardiography. 2002;19(2):109-14. doi: 10.1046/j.1540-8175.2002.00109.x.

71. Abdelmoneim SS, Dhoble A, Bernier M, Moir S, Hagen ME, Ness SA, et al. Absolute Myocardial Blood Flow Determination using Real-Time Myocardial Contrast Echocardiography during Adenosine Stress: Comparison with Single-Photon Emission Computed Tomography. Heart. 2009;95(20):1662-8. doi: 10.1136/hrt.2009.169268.

72. Wei K, Ragosta M, Thorpe J, Coggins M, Moos S, Kaul S. Noninvasive Quantification of Coronary Blood Flow Reserve in Humans using Myocardial Contrast Echocardiography. Circulation. 2001;103(21):2560-5. doi: 10.1161/01.cir.103.21.2560.

73. Peltier M, Vancraeynest D, Pasquet A, Ay T, Roelants V, D'hondt AM, et al. Assessment of the Physiologic Significance of Coronary Disease with Dipyridamole Real-Time Myocardial Contrast Echocardiography. Comparison with Technetium-99m Sestamibi Single-Photon Emission Computed Tomography and Quantitative Coronary Angiography. J Am Coll Cardiol. 2004;43(2):257-64. doi: 10.1016/j.jacc.2003.07.040.

74. Miszalski-Jamka T, Kuntz-Hehner S, Schmidt H, Peter D, Miszalski-Jamka K, Hammerstingl C, et al. Myocardial Contrast Echocardiography Enhances Long-Term Prognostic Value of Supine Bicycle Stress Two-Dimensional Echocardiography. J Am Soc Echocardiogr. 2009;22(11):1220-7. doi: 10.1016/j.echo.2009.07.020.

75. Lipiec P, Wejner-Mik P, Krzemińska-Pakuła M, Kuśmierek J, Płachcińska A, Szumiński R, et al. Accelerated Stress Real-Time Myocardial Contrast Echocardiography for the Detection of Coronary Artery Disease: Comparison with 99mTc Single Photon Emission Computed Tomography. J Am Soc Echocardiogr. 2008;21(8):941-7. doi: 10.1016/j.echo.2008.02.004.

76. Lønnebakken MT, Bleie O, Strand E, Staal EM, Nygård OK, Gerdts E. Myocardial Contrast Echocardiography in Assessment of Stable Coronary Artery Disease at Intermediate Dobutamine-Induced Stress Level. Echocardiography. 2009;26(1):52-60. doi: 10.1111/j.1540-8175.2008.00738.x.

77. Leong-Poi H, Rim SJ, Le DE, Fisher NG, Wei K, Kaul S. Perfusion versus Function: The Ischemic Cascade in Demand Ischemia: Implications of Single-Vessel versus Multivessel Stenosis. Circulation. 2002;105(8):987-92. doi: 10.1161/hc0802.104326.

78. Abdelmoneim SS, Dhoble A, Bernier M, Erwin PJ, Korosoglou G, Senior R, et al. Quantitative Myocardial Contrast Echocardiography during Pharmacological Stress for Diagnosis of Coronary Artery Disease: A Systematic Review and Meta-Analysis of Diagnostic Accuracy Studies. Eur J Echocardiogr. 2009;10(7):813-25. doi: 10.1093/ejechocard/jep084.

79. Osório AF, Tsutsui JM, Kowatsch I, Guerra VC, Ramires JA, Lemos PA, et al. Evaluation of Blood Flow Reserve in Left Anterior Descending Coronary Artery Territory by Quantitative Myocardial Contrast and Doppler Echocardiography. J Am Soc Echocardiogr. 2007;20(6):709-16. doi: 10.1016/j.echo.2006.11.016.

80. Mulvagh SL, DeMaria AN, Feinstein SB, Burns PN, Kaul S, Miller JG, et al. Contrast Echocardiography: Current and Future Applications. J Am Soc Echocardiogr. 2000;13(4):331-42. doi: 10.1067/mje.2000.105462.

81. Senior R, Becher H, Monaghan M, Agati L, Zamorano J, Vanoverschelde JL, et al. Clinical Practice of Contrast Echocardiography: Recommendation by the European Association of Cardiovascular Imaging (EACVI) 2017. Eur Heart J Cardiovasc Imaging. 2017;18(11):1205-1205af. doi: 10.1093/ehjci/jex182.

82. Hampson R, Senior R, Ring L, Robinson S, Augustine DX, Becher H, et al. Contrast Echocardiography: A Practical Guideline from the British Society of Echocardiography. Echo Res Pract. 2023;10(1):23. doi: 10.1186/s44156-023-00034-9.

83. Bhatia VK, Senior R. Contrast Echocardiography: Evidence for Clinical Use. J Am Soc Echocardiogr. 2008;21(5):409-16. doi: 10.1016/j.echo.2008.01.018.

84. Sharples L, Hughes V, Crean A, Dyer M, Buxton M, Goldsmith K, et al. Cost-Effectiveness of Functional Cardiac Testing in the Diagnosis and Management of Coronary Artery Disease: A Randomised Controlled Trial. The CECaT trial. Health Technol Assess. 2007;11(49):iii-iv, ix-115. doi: 10.3310/hta11490.

85. Tardif JC, Dore A, Chan KL, Fagan S, Honos G, Marcotte F, et al. Economic Impact of Contrast Stress Echocardiography on the Diagnosis and Initial Treatment of Patients with Suspected Coronary Artery Disease. J Am Soc Echocardiogr. 2002;15(11):1335-45. doi: 10.1067/mje.2002.125287.

86. Porter TR, Xie F. Myocardial Perfusion Imaging with Contrast Ultrasound. JACC Cardiovasc Imaging. 2010;3(2):176-87. doi: 10.1016/j.jcmg.2009.09.024.

87. Dijkmans PA, Senior R, Becher H, Porter TR, Wei K, Visser CA, et al. Myocardial Contrast Echocardiography Evolving as a Clinically Feasible Technique for Accurate, Rapid, and Safe Assessment of Myocardial Perfusion: The Evidence So Far. J Am Coll Cardiol. 2006;48(11):2168-77. doi: 10.1016/j.jacc.2006.05.079.

88. Kowatsch I, Tsutsui JM, Osório AF, Uchida AH, Machiori GG, Lopes ML, et al. Head-to-Head Comparison of Dobutamine and Adenosine Stress Real-Time Myocardial Perfusion Echocardiography for the Detection of Coronary Artery Disease. J Am Soc Echocardiogr. 2007;20(9):1109-17. doi: 10.1016/j.echo.2007.02.008.

89. Tsutsui JM, Elhendy A, Anderson JR, Xie F, McGrain AC, Porter TR. Prognostic Value of Dobutamine Stress Myocardial Contrast Perfusion Echocardiography. Circulation. 2005;112(10):1444-50. doi: 10.1161/CIRCULATIONAHA.105.537134.

90. Anantharam B, Janardhanan R, Hayat S, Hickman M, Chahal N, Bassett P, et al. Coronary Flow Reserve Assessed by Myocardial Contrast Echocardiography Predicts Mortality in Patients with Heart Failure. Eur J Echocardiogr. 2011;12(1):69-75. doi: 10.1093/ejechocard/jeq109.

91. Di Bello V, Giorgi D, Pedrinelli R, Talini E, Palagi C, Delle Donne MG, et al. Early Impairment of Myocardial Blood Flow Reserve in Men with Essential Hypertension: A Quantitative Myocardial Contrast Echocardiography Study. J Am Soc Echocardiogr. 2004;17(10):1037-43. doi: 10.1016/j.echo.2004.05.017.

92. Senior R, Lepper W, Pasquet A, Chung G, Hoffman R, Vanoverschelde JL, et al. Myocardial Perfusion Assessment in Patients with Medium Probability of Coronary Artery Disease and no Prior Myocardial Infarction: Comparison of Myocardial Contrast Echocardiography with 99mTc Single-Photon Emission Computed Tomography. Am Heart J. 2004;147(6):1100-5. doi: 10.1016/j.ahj.2003.12.030.

93. Senior R, Moreo A, Gaibazzi N, Agati L, Tiemann K, Shivalkar B, et al. Comparison of Sulfur Hexafluoride Microbubble (SonoVue)-enhanced Myocardial Contrast Echocardiography with Gated Single-Photon Emission Computed Tomography for Detection of Significant Coronary Artery Disease: A Large European Multicenter Study. J Am Coll Cardiol. 2013;62(15):1353-61. doi: 10.1016/j.jacc.2013.04.082.

94. Hayat SA, Dwivedi G, Jacobsen A, Lim TK, Kinsey C, Senior R. Effects of Left Bundle-Branch Block on Cardiac Structure, Function, Perfusion, and Perfusion Reserve: Implications for Myocardial Contrast Echocardiography versus Radionuclide Perfusion Imaging for the Detection of Coronary Artery Disease. Circulation. 2008;117(14):1832-41. doi: 10.1161/CIRCULATIONAHA.107.726711.

95. Senior R, Monaghan M, Main ML, Zamorano JL, Tiemann K, Agati L, et al. Detection of Coronary Artery Disease with Perfusion Stress Echocardiography Using a Novel Ultrasound Imaging Agent: Two Phase 3 International Trials in Comparison with Radionuclide Perfusion Imaging. Eur J Echocardiogr. 2009;10(1):26-35. doi: 10.1093/ejechocard/jen321.

96. Shimoni S, Frangogiannis NG, Aggeli CJ, Shan K, Quinones MA, Espada R, et al. Microvascular Structural Correlates of Myocardial Contrast Echocardiography in Patients with Coronary Artery Disease and Left Ventricular Dysfunction: Implications for the Assessment of Myocardial Hibernation. Circulation. 2002;106(8):950-6. doi: 10.1161/01.cir.0000026395.19594.43.

97. Edvardsen T, Asch FM, Davidson B, Delgado V, DeMaria A, Dilsizian V, et al. Non-Invasive Imaging in Coronary Syndromes: Recommendations of the European Association of Cardiovascular Imaging and the American Society of Echocardiography, in collaboration with the American Society of Nuclear Cardiology, Society of Cardiovascular Computed Tomography, and Society for Cardiovascular Magnetic Resonance. Eur Heart J Cardiovasc Imaging. 2022;23(2):e6-e33. doi: 10.1093/ehjci/jeab244.

98. Schinkel AF, Bax JJ, Poldermans D, Elhendy A, Ferrari R, Rahimtoola SH. Hibernating Myocardium: Diagnosis and Patient Outcomes. Curr Probl Cardiol. 2007;32(7):375-410. doi: 10.1016/j.cpcardiol.2007.04.001.

99. De Filippi CR, Willett DL, Irani WN, Eichhorn EJ, Velasco CE, Grayburn PA. Comparison of Myocardial Contrast Echocardiography and Low-Dose Dobutamine Stress Echocardiography in Predicting Recovery of Left Ventricular Function after Coronary Revascularization in Chronic Ischemic Heart Disease. Circulation. 1995;92(10):2863-8. doi: 10.1161/01.cir.92.10.2863.

100. Nagueh SF, Vaduganathan P, Ali N, Blaustein A, Verani MS, Winters WL Jr, et al. Identification of Hibernating Myocardium: Comparative Accuracy of Myocardial Contrast Echocardiography, Rest-Redistribution Thallium-201 Tomography and Dobutamine Echocardiography. J Am Coll Cardiol. 1997;29(5):985-93. doi: 10.1016/s0735-1097(97)00001-6.

101. Meza MF, Ramee S, Collins T, Stapleton D, Milani RV, Murgo JP, et al. Knowledge of Perfusion and Contractile Reserve Improves the Predictive Value of Recovery of Regional Myocardial Function Postrevascularization: A Study using the Combination of Myocardial Contrast Echocardiography and Dobutamine Echocardiography. Circulation. 1997;96(10):3459-65. doi: 10.1161/01.cir.96.10.3459.

102. Sbano JC, Tsutsui JM, Andrade JL, Nicolau JC, Meneghetti JC, Ramires JF, et al. Detection of Functional Recovery Using Low-Dose Dobutamine and Myocardial Contrast Echocardiography after Acute Myocardial Infarction Treated with Successful Thrombolytic Therapy. Echocardiography. 2005;22(6):496-502. doi: 10.1111/j.1540-8175.2005.04007.x.

103. Li L, Hu N, Li L, Li L. Low-Dose Dobutamine Stress Myocardial Contrast Echocardiography for Evaluating Myocardial Microcirculation Perfusion and Predicting Long-Term Prognosis in ST-Segment Elevation Myocardial Infarction after Percutaneous Coronary Intervention. J Cardiothorac Surg. 2025;20(1):125. doi: 10.1186/s13019-024-03216-6.

104. Fernandes DR, Tsutsui JM, Bocchi EA, César LA, Sbano JC, Ramires JA, et al. Qualitative and Quantitative Real Time Myocardial Contrast Echocardiography for Detecting Hibernating Myocardium. Echocardiography. 2011;28(3):342-9. doi: 10.1111/j.1540-8175.2010.01317.x.

105. Shimoni S, Frangogiannis NG, Aggeli CJ, Shan K, Verani MS, Quinones MA, et al. Identification of Hibernating Myocardium with Quantitative Intravenous Myocardial Contrast Echocardiography: Comparison with Dobutamine Echocardiography and Thallium-201 Scintigraphy. Circulation. 2003;107(4):538-44. doi: 10.1161/01.cir.0000047211.53448.12.

106. Wu X, Chen L, Yang Y. Myocardial Contrast Echocardiography Predicts Major Adverse Cardiovascular and Cerebrovascular Events in the Population after Percutaneous Coronary Intervention-A Systematic Review and Meta-Analysis. Cardiovasc Diagn Ther. 2025;15(4):802-19. doi: 10.21037/cdt-2024-664.

107. Chen F, Weng W, Yang D, Wang X, Zhou Y. Myocardial Contrast Echocardiography Evaluation of Coronary Microvascular Dysfunction to Predict MACEs in Patients with Heart Failure with Preserved Ejection Fraction Follow-Up. BMC Cardiovasc Disord. 2024;24(1):496. doi: 10.1186/s12872-024-04173-7.

108. Gvinianidze L, Toulemonde M, Hampson R, Huang B, Bioh G, Wakefield LA, et al. Ultrafast Myocardial Contrast Echocardiography for the Assessment of Coronary Artery Disease: First In-Human Study. Circ Cardiovasc Imaging. 2024;17(10):e017267. doi: 10.1161/CIRCIMAGING.124.017267.

109. Becher H, Helfen A, Michels G, Gaibazzi N, Senior R, Dietrich CF. How to Perform Cardiac Contrast-Enhanced Ultrasound (cCEUS): Part I. Diagnostics. 2025;15(14):1743. doi: 10.3390/diagnostics15141743.

110. Saba L, Saam T, Jäger HR, Yuan C, Hatsukami TS, Saloner D, et al. Imaging Biomarkers of Vulnerable Carotid Plaques for Stroke Risk Prediction and their Potential Clinical Implications. Lancet Neurol. 2019;18(6):559-72. doi: 10.1016/S1474-4422(19)30035-3.

111. Kopyto E, Czeczelewski M, Mikos E, Stępniak K, Kopyto M, Matuszek M, et al. Contrast-Enhanced Ultrasound Feasibility in Assessing Carotid Plaque Vulnerability-Narrative Review. J Clin Med. 2023;12(19):6416. doi: 10.3390/jcm12196416.

112. Chen J, Stavropoulos SW. Management of Endoleaks. Semin Intervent Radiol. 2015;32(3):259-64. doi: 10.1055/s-0035-1556825.

113. Kapetanios D, Kontopodis N, Mavridis D, McWilliams RG, Giannoukas AD, Antoniou GA. Meta-Analysis of the Accuracy of Contrast-Enhanced Ultrasound for the Detection of Endoleak after Endovascular Aneurysm Repair. J Vasc Surg. 2019;69(1):280-294.e6. doi: 10.1016/j.jvs.2018.07.044.

114. Strom JB, Mulvagh SL, Porter TR, Wei K, Stout JL, Main ML. Contemporary Safety of Ultrasound Enhancing Agents in a Nationwide Analysis. J Am Heart Assoc. 2025;14(10):e039480. doi: 10.1161/JAHA.124.039480.

115. Kutty S, Xiao Y, Olson J, Xie F, Danford DA, Erickson CC, et al. Safety and Efficacy of Cardiac Ultrasound Contrast in Children and Adolescents for Resting and Stress Echocardiography. J Am Soc Echocardiogr. 2016;29(7):655-62. doi: 10.1016/j.echo.2016.02.019.

116. Lopez L, Saurers DL, Barker PCA, Cohen MS, Colan SD, Dwyer J, et al. Guidelines for Performing a Comprehensive Pediatric Transthoracic Echocardiogram: Recommendations from the American Society of Echocardiography. J Am Soc Echocardiogr. 2024;37(2):119-170. doi: 10.1016/j.echo.2023.11.015.

117. Hwang M, Back SJ, Didier RA, Lorenz N, Morgan TA, Poznick L, et al. Pediatric Contrast-Enhanced Ultrasound: Optimization of Techniques and Dosing. Pediatr Radiol. 2021;51(12):2147-60. doi: 10.1007/s00247-020-04812-z.

118. Sabati A, Wang S, Shivaram P, Hawley J, Thompson D, Erenberg F, et al. A Phase 4, Open-Label, Non-Randomized, Multicenter Study to Evaluate Efficacy (and Safety) of Intravenous Administration of Optison for Contrast-Enhanced Echocardiography in Pediatric Patients. Pediatr Cardiol. 2026;47(5):2052-66. doi: 10.1007/s00246-025-03993-4.

119. Mathias W Jr, Arrieta SR, Tavares GMP, Sbano JCN, Tsutsui JM, Kutty S, et al. Successful Recanalization of Thrombotic Occlusion in Pulmonary Artery Stent using Sonothrombolysis. CASE. 2019;3(1):14-7. doi: 10.1016/j.case.2018.11.006.

120. Parker JM, Weller MW, Feinstein LM, Adams RJ, Main ML, Grayburn PA, et al. Safety of Ultrasound Contrast Agents in Patients with Known or Suspected Cardiac Shunts. Am J Cardiol. 2013;112(7):1039-45. doi: 10.1016/j.amjcard.2013.05.042.

121. Muskula PR, Main ML. Safety with Echocardiographic Contrast Agents. Circ Cardiovasc Imaging. 2017;10(4):e005459. doi: 10.1161/CIRCIMAGING.116.005459.

122. Darge K, Back SJ, Bulas DI, Feinstein SB, Ntoulia A, Volberg FM, et al. Pediatric Contrast-Enhanced Ultrasound: Shedding Light on the Pursuit of Approval in the United States. Pediatr Radiol. 2021;51(12):2128-38. doi: 10.1007/s00247-021-05102-y.

123. Mitchell C, Rahko PS, Blauwet LA, Canaday B, Finstuen JA, Foster MC, et al. Guidelines for Performing a Comprehensive Transthoracic Echocardiographic Examination in Adults: Recommendations from the American Society of Echocardiography. J Am Soc Echocardiogr. 2019;32(1):1-64. doi: 10.1016/j.echo.2018.06.004.

124. Rodríguez-Roisin R, Krowka MJ. Hepatopulmonary Syndrome--A Liver-Induced Lung Vascular Disorder. N Engl J Med. 2008;358(22):2378-87. doi: 10.1056/NEJMra0707185.

125. Zaidi SJ, Adhikari RR, Patel DR, Cui VW, Javois AJ, Roberson DA. Saline Contrast Transesophageal Echocardiography in Fontan Patients: Assessment of the Presence, Type, and Size of Right to Left Shunts. Pediatr Cardiol. 2019 Aug;40(6):1199-207. doi: 10.1007/s00246-019-02132-0.

126. Gupta SK, Shetkar SS, Ramakrishnan S, Kothari SS. Saline Contrast Echocardiography in the Era of Multimodality Imaging--Importance of "Bubbling It Right". Echocardiography. 2015;32(11):1707-19. doi: 10.1111/echo.13035.

127. Porter TR, Shillcutt SK, Adams MS, Desjardins G, Glas KE, Olson JJ, et al. Guidelines for the Use of Echocardiography as a Monitor for Therapeutic Intervention in Adults: A Report from the American Society of Echocardiography. J Am Soc Echocardiogr. 2015;28(1):40-56. doi: 10.1016/j.echo.2014.09.009.

128. Porter TR, Feinstein SB, Senior R, Mulvagh SL, Nihoyannopoulos P, Strom JB, et al. CEUS Cardiac Exam Protocols International Contrast Ultrasound Society (ICUS) recommendations. Echo Res Pract. 2022;9(1):7. doi: 10.1186/s44156-022-00008-3.

129. Kusnetzky LL, Khalid A, Khumri TM, Moe TG, Jones PG, Main ML. Acute Mortality in Hospitalized Patients Undergoing Echocardiography with and without an Ultrasound Contrast Agent: Results in 18,671 Consecutive Studies. J Am Coll Cardiol. 2008;51(17):1704-6. doi: 10.1016/j.jacc.2008.03.006.

130. Wei K, Mulvagh SL, Carson L, Davidoff R, Gabriel R, Grimm RA, et al. The Safety of deFinity and Optison for Ultrasound Image Enhancement: A Retrospective Analysis of 78,383 Administered Contrast Doses. J Am Soc Echocardiogr. 2008;21(11):1202-6. doi: 10.1016/j.echo.2008.07.019.

131. Furtado RG, Rassi DDC, Melato LH, Oliveira ACR, Nunes PM, Baccelli PE, et al. Safety of SF6(SonoVue®) Contrast Agent on Pharmacological Stress Echocardiogram. Arq Bras Cardiol. 2021;117(6):1170-8. doi: 10.36660/abc.20200475.

132. Abdelmoneim SS, Bernier M, Scott CG, Dhoble A, Ness SA, Hagen ME, et al. Safety of Contrast Agent Use During Stress Echocardiography in Patients with Elevated Right Ventricular Systolic Pressure: A Cohort Study. Circ Cardiovasc Imaging. 2010;3(3):240-8. doi: 10.1161/CIRCIMAGING.109.895029.

133. Wever-Pinzon O, Suma V, Ahuja A, Romero J, Sareen N, Henry SA, et al. Safety of Echocardiographic Contrast in Hospitalized Patients with Pulmonary Hypertension: A Multi-Center Study. Eur Heart J Cardiovasc Imaging. 2012;13(10):857-62. doi: 10.1093/ehjci/jes057.

134. Lindner JR, Belcik T, Main ML, Montanaro A, Mulvagh SL, Olson J, et al. Expert Consensus Statement from the American Society of Echocardiography on Hypersensitivity Reactions to Ultrasound Enhancing Agents in Patients with Allergy to Polyethylene Glycol. J Am Soc Echocardiogr. 2021;34(7):707-8. doi: 10.1016/j.echo.2021.05.002.

135. Yong Y, Wu D, Fernandes V, Kopelen HA, Shimoni S, Nagueh SF, et al. Diagnostic Accuracy and Cost-Effectiveness of Contrast Echocardiography on Evaluation of cardiac Function in Technically Very Difficult Patients in the Intensive Care Unit. Am J Cardiol. 2002;89(6):711-8. doi: 10.1016/s0002-9149(01)02344-x.

136. Costa JM, Tsutsui JM, Nozawa E, Morhy SS, Andrade JL, Ramires JF, et al. Contrast Echocardiography Can Save Nondiagnostic Exams in Mechanically Ventilated Patients. Echocardiography. 2005;22(5):389-94. doi: 10.1111/j.1540-8175.2005.03176.x.

137. Reilly JP, Tunick PA, Timmermans RJ, Stein B, Rosenzweig BP, Kronzon I. Contrast Echocardiography Clarifies Uninterpretable Wall Motion in Intensive Care Unit Patients. J Am Coll Cardiol. 2000;35(2):485-90. doi: 10.1016/s0735-1097(99)00558-6.

138. Siebelink HM, Scholte AJ, van de Veire NR, Holman ER, Nucifora G, van der Wall EE, et al. Value of Contrast Echocardiography for Left Ventricular Thrombus Detection Postinfarction and Impact on Antithrombotic Therapy. Coron Artery Dis. 2009;20(7):462-6. doi: 10.1097/mca.0b013e328330d58f.

139. Tong KL, Kaul S, Wang XQ, Rinkevich D, Kalvaitis S, Belcik T, et al. Myocardial Contrast Echocardiography Versus Thrombolysis In Myocardial Infarction Score in Patients Presenting to the Emergency Department with Chest Pain and a Nondiagnostic Electrocardiogram. J Am Coll Cardiol. 2005;46(5):920-7. doi: 10.1016/j.jacc.2005.03.076.

140. Nayyar S, Magalski A, Khumri TM, Idupulapati M, Stoner CN, Kusnetzky LL, et al. Contrast Administration Reduces Interobserver Variability in Determination of Left Ventricular Ejection Fraction in Patients with Left Ventricular Dysfunction and Good Baseline Endocardial Border Delineation. Am J Cardiol. 2006;98(8):1110-4. doi: 10.1016/j.amjcard.2006.05.038.

141. Shah BN, Zacharias K, Pabla JS, Karogiannis N, Calicchio F, Balaji G, et al. The clinical Impact of Contemporary Stress Echocardiography in Morbid Obesity for the Assessment of Coronary Artery Disease. Heart. 2016;102(5):370-5. doi: 10.1136/heartjnl-2015-308796.

142. Jeetley P, Burden L, Stoykova B, Senior R. Clinical and Economic Impact of Stress Echocardiography Compared with Exercise Electrocardiography in Patients with Suspected Acute Coronary Syndrome but Negative Troponin: A Prospective Randomized Controlled Study. Eur Heart J. 2007;28(2):204-11. doi: 10.1093/eurheartj/ehl444.

143. Xie F, Lof J, Everbach C, He A, Bennett RM, Matsunaga T, et al. Treatment of Acute Intravascular Thrombi with Diagnostic Ultrasound and Intravenous Microbubbles. JACC Cardiovasc Imaging. 2009;2(4):511-8. doi: 10.1016/j.jcmg.2009.02.002.

144. Belcik JT, Mott BH, Xie A, Zhao Y, Kim S, Lindner NJ, et al. Augmentation of Limb Perfusion and Reversal of Tissue Ischemia Produced by Ultrasound-Mediated Microbubble Cavitation. Circ Cardiovasc Imaging. 2015;8(4):e002979. doi: 10.1161/CIRCIMAGING.114.002979.

145. Mathias W Jr, Tsutsui JM, Tavares BG, Fava AM, Aguiar MOD, Borges BC, et al. Sonothrombolysis in ST-Segment Elevation Myocardial Infarction Treated with Primary Percutaneous Coronary Intervention. J Am Coll Cardiol. 2019;73(22):2832-42. doi: 10.1016/j.jacc.2019.03.006.

146. Mathias W Jr, Tsutsui JM, Tavares BG, Xie F, Aguiar MO, Garcia DR, et al. Diagnostic Ultrasound Impulses Improve Microvascular Flow in Patients with STEMI Receiving Intravenous Microbubbles. J Am Coll Cardiol. 2016;67(21):2506-15. doi: 10.1016/j.jacc.2016.03.542.

147. Aguiar MOD, Tavares BG, Tsutsui JM, Fava AM, Borges BC, Oliveira MT Jr, et al. Sonothrombolysis Improves Myocardial Dynamics and Microvascular Obstruction Preventing Left Ventricular Remodeling in Patients with ST Elevation Myocardial Infarction. Circ Cardiovasc Imaging. 2020;13(4):e009536. doi: 10.1161/CIRCIMAGING.119.009536.

148. Le Bihan D. Therapeutic Echocardiography. Arq Bras Cardiol. 2022;118(4):766-7. doi: 10.36660/abc.20220014.

149. Tavares BG, Aguiar MO, Tsutsui J, Oliveira M, Soeiro AM, Nicolau J, et al. Sonothrombolysis Promotes Improvement in Left Ventricular Wall Motion and Perfusion Scores after Acute Myocardial Infarction. Arq Bras Cardiol. 2022;118(4):756-65. doi: 10.36660/abc.20200651.

150. Chiang HP, Aguiar MOD, Tavares BG, Rosa VEE, Gomes SB, Oliveira MT Jr, et al. The Impact of Sonothrombolysis on Left Ventricular Diastolic Function and Left Atrial Mechanics Preventing Left Atrial Remodeling in Patients with ST Elevation Acute Myocardial Infarction. J Am Soc Echocardiogr. 2023;36(5):504-13. doi: 10.1016/j.echo.2022.12.010.

151. Nahrendorf M, Sosnovik DE, French BA, Swirski FK, Bengel F, Sadeghi MM, et al. Multimodality Cardiovascular Molecular Imaging, Part II. Circ Cardiovasc Imaging. 2009;2(1):56-70. doi: 10.1161/CIRCIMAGING.108.839092.

152. Sinusas AJ, Bengel F, Nahrendorf M, Epstein FH, Wu JC, Villanueva FS, et al. Multimodality Cardiovascular Molecular Imaging, Part I. Circ Cardiovasc Imaging. 2008;1(3):244-56. doi: 10.1161/CIRCIMAGING.108.824359.

153. Lindner JR. Molecular Imaging of Cardiovascular Disease with Contrast-Enhanced Ultrasonography. Nat Rev Cardiol. 2009;6(7):475-81. doi: 10.1038/nrcardio.2009.77.

154. Maul TM, Dudgeon DD, Beste MT, Hammer DA, Lazo JS, Villanueva FS, et al. Optimization of Ultrasound Contrast Agents with Computational Models to Improve Selection of Ligands and Binding Strength. Biotechnol Bioeng. 2010;107(5):854-64. doi: 10.1002/bit.22857.

155. Mayer CR, Bekeredjian R. Ultrasonic Gene and Drug Delivery to the Cardiovascular System. Adv Drug Deliv Rev. 2008;60(10):1177-92. doi: 10.1016/j.addr.2008.03.004.

156. Xie A, Belcik T, Qi Y, Morgan TK, Champaneri SA, Taylor S, et al. Ultrasound-Mediated Vascular Gene Transfection by Cavitation of Endothelial-Targeted Cationic Microbubbles. JACC Cardiovasc Imaging. 2012;5(12):1253-62. doi: 10.1016/j.jcmg.2012.05.017.

157. Sun L, Huang CW, Wu J, Chen KJ, Li SH, Weisel RD, et al. The Use of Cationic Microbubbles to Improve Ultrasound-Targeted Gene Delivery to the Ischemic Myocardium. Biomaterials. 2013;34(8):2107-16. doi: 10.1016/j.biomaterials.2012.11.041.

158. Chen S, Shohet RV, Bekeredjian R, Frenkel P, Grayburn PA. Optimization of Ultrasound Parameters for Cardiac Gene Delivery of Adenoviral or Plasmid Deoxyribonucleic Acid by Ultrasound-Targeted Microbubble Destruction. J Am Coll Cardiol. 2003;42(2):301-8. doi: 10.1016/s0735-1097(03)00627-2.

159. Bekeredjian R, Chen S, Frenkel PA, Grayburn PA, Shohet RV. Ultrasound-Targeted Microbubble Destruction can Repeatedly Direct Highly Specific Plasmid Expression to the Heart. Circulation. 2003;108(8):1022-6. doi: 10.1161/01.CIR.0000084535.35435.AE.

160. Cao WJ, Rosenblat JD, Roth NC, Kuliszewski MA, Matkar PN, Rudenko D, et al. Therapeutic Angiogenesis by Ultrasound-Mediated MicroRNA-126-3p Delivery. Arterioscler Thromb Vasc Biol. 2015;35(11):2401-11. doi: 10.1161/ATVBAHA.115.306506.

161. Fujii H, Li SH, Wu J, Miyagi Y, Yau TM, Rakowski H, et al. Repeated and Targeted Transfer of Angiogenic Plasmids Into the Infarcted Rat Heart via Ultrasound Targeted Microbubble Destruction Enhances Cardiac Repair. Eur Heart J. 2011;32(16):2075-84. doi: 10.1093/eurheartj/ehq475.

162. Maron BJ, Ommen SR, Semsarian C, Spirito P, Olivotto I, Maron MS. Hypertrophic Cardiomyopathy: Present and Future, with Translation Into Contemporary Cardiovascular Medicine. J Am Coll Cardiol. 2014;64(1):83-99. doi: 10.1016/j.jacc.2014.05.003.

163. Pelliccia F, Niccoli G, Gragnano F, Limongelli G, Moscarella E, Andò G, et al. Alcohol Septal Ablation for Hypertrophic Obstructive Cardiomyopathy: A Contemporary Reappraisal. EuroIntervention. 2019;15(5):411-7. doi: 10.4244/EIJ-D-18-00959.

164. Ommen SR, Ho CY, Asif IM, Balaji S, Burke MA, Day SM, et al. 2024 AHA/ACC/AMSSM/HRS/PACES/SCMR Guideline for the Management of Hypertrophic Cardiomyopathy: A Report of the American Heart Association/American College of Cardiology Joint Committee on Clinical Practice Guidelines. Circulation. 2024;149(23):e1239-e1311. doi: 10.1161/CIR.0000000000001250.

165. Hsieh TC, Patel K. Echocardiography-Guided Alcohol Septal Ablation for Hypertrophic Obstructive Cardiomyopathy. Anesth Analg. 2011;113(1):44-6. doi: 10.1213/ANE.0b013e31821a9fa6.

166. Sorajja P, Valeti U, Nishimura RA, Ommen SR, Rihal CS, Gersh BJ, et al. Outcome of Alcohol Septal Ablation for Obstructive Hypertrophic Cardiomyopathy. Circulation. 2008;118(2):131-9. doi: 10.1161/CIRCULATIONAHA.107.738740.

167. Faber L, Welge D, Fassbender D, Buschler H, Schmidt HK, Horstkotte D. Percutaneous Septal Ablation in Hypertrophic Obstructive Cardiomyopathy: Current Status. EuroIntervention. 2005;1(3):358-66.

168. Agarwal S, Tuzcu EM, Desai MY, Smedira N, Lever HM, Lytle BW, et al. Updated Meta-Analysis of Septal Alcohol Ablation versus Myectomy for Hypertrophic Cardiomyopathy. J Am Coll Cardiol. 2010;55(8):823-34. doi: 10.1016/j.jacc.2009.09.047.

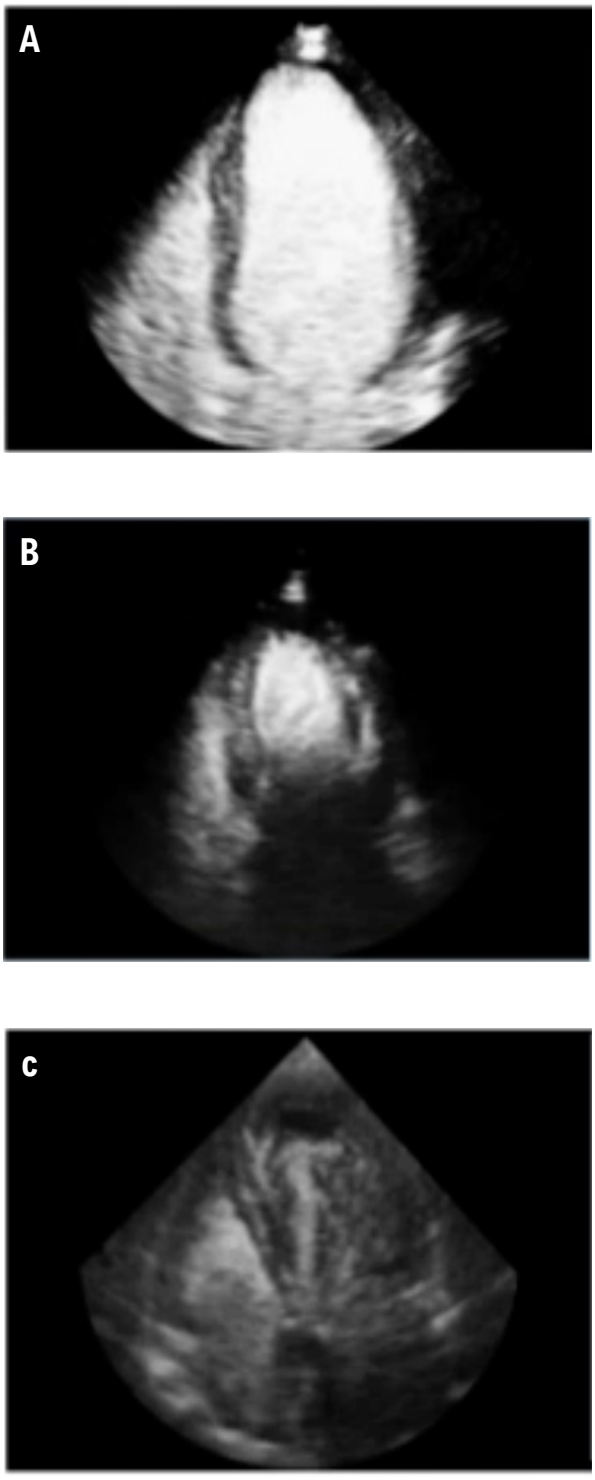
169. Catino AB, Ross A, Suksaranjit P, Wilson BD. Improved Thrombus Assessment by Transesophageal Echocardiography: The DOLOP (Detection of Left Atrial Appendage Thrombosis Utilizing Optison) Study. J Am Soc Echocardiogr. 2021;34(8):916-7. doi: 10.1016/j.echo.2021.03.012.

170. Ebelt H, Offhaus A, Wiora M, Roehl P, Schwenzky A, Weida A, et al. Impact of Ultrasound Contrast Agent on the Detection of Thrombi during Transoesophageal Echocardiography. Open Heart. 2019;6(2):e001024. doi: 10.1136/openhrt-2019-001024.

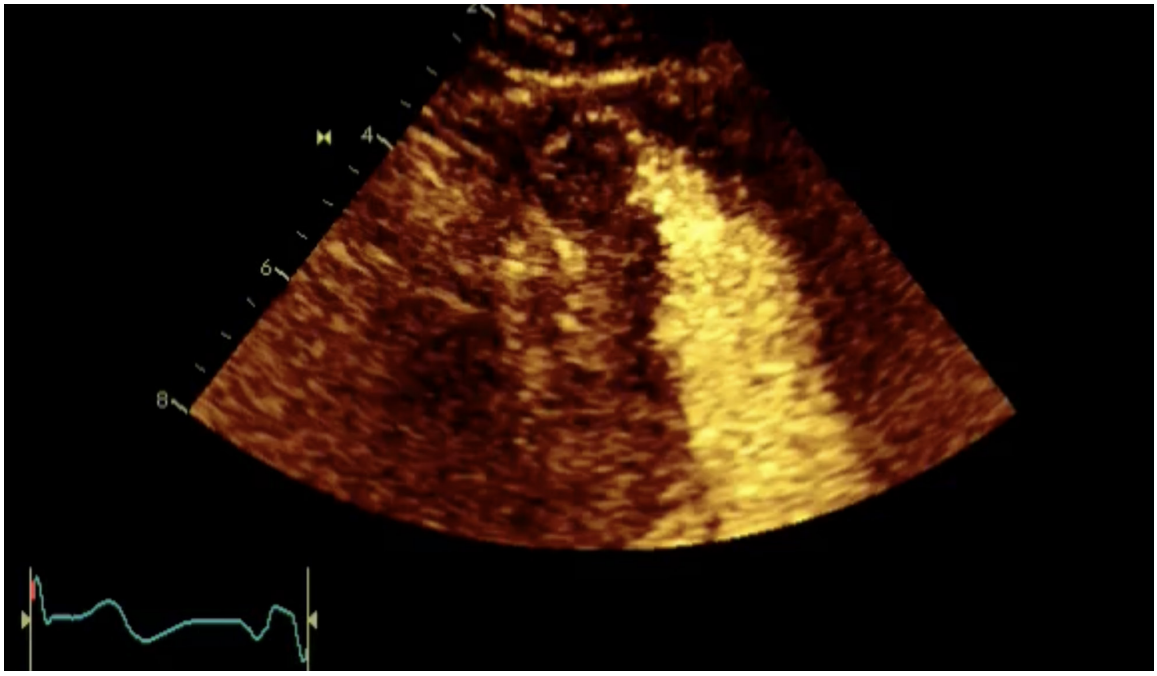
171. Jain R, Wessly P, Saric M, Richardson K, Garcia-Sayan E, Addetia K, et al. Multimodality Imaging in Evaluating and Guiding Percutaneous Left Atrial Appendage Occlusion. J Am Soc Echocardiogr. 2025;38(12):1198-218. doi: 10.1016/j.echo.2025.07.009.

172. Strom JB, Appis A, Barr RG, Chammas MC, Clevert DA, Darge K, et al. Multi-Societal Expert Consensus Statement on the Safe Administration of Ultrasound Contrast Agents. Echo Res Pract. 2025;12(1):4. doi: 10.1186/s44156-024-00068-7.

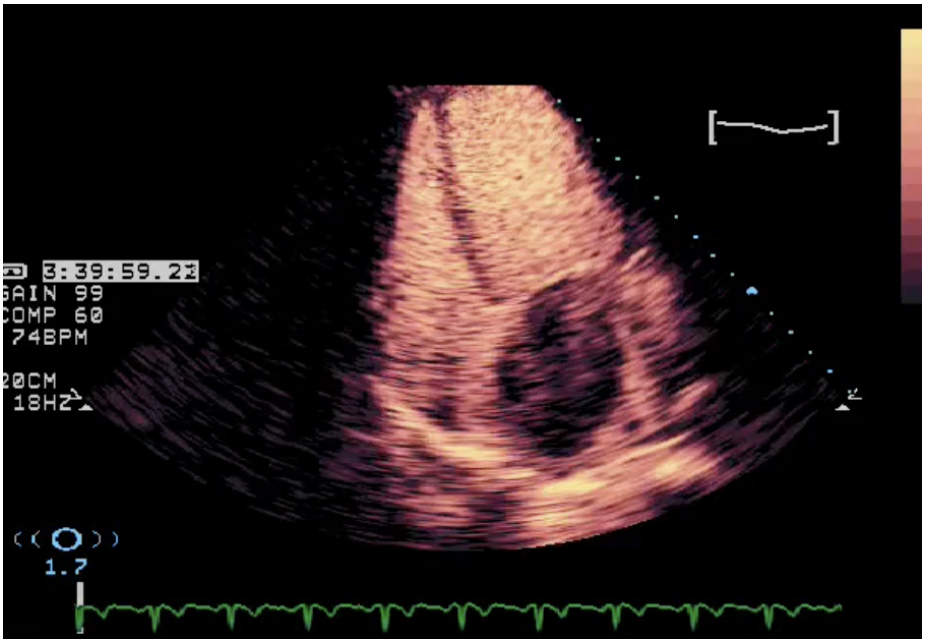
Supplementary material



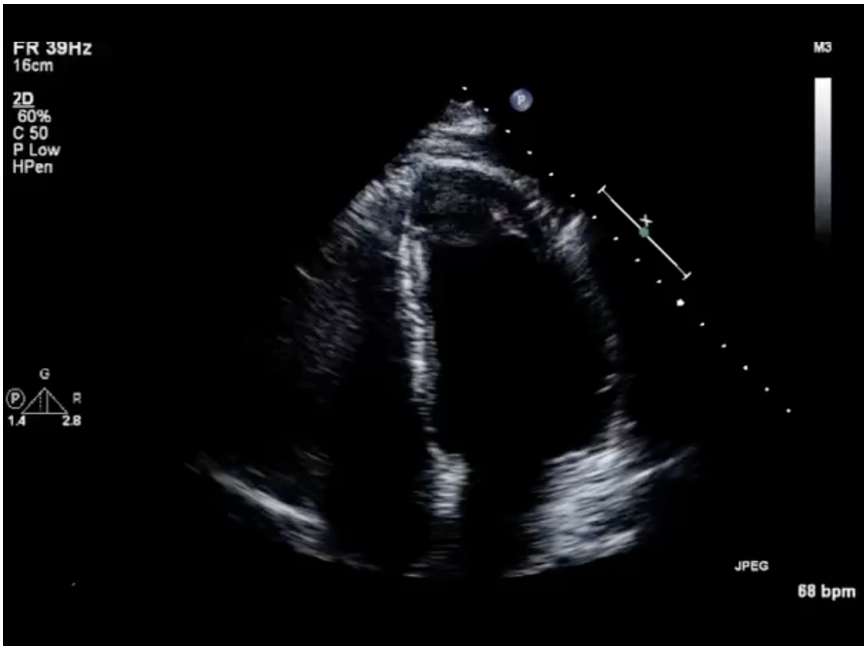
Supplementary Video 1 – *Demonstrating the ideal opacification conditions (A), attenuation (B) and apical swirl (C). Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_01.mp4*



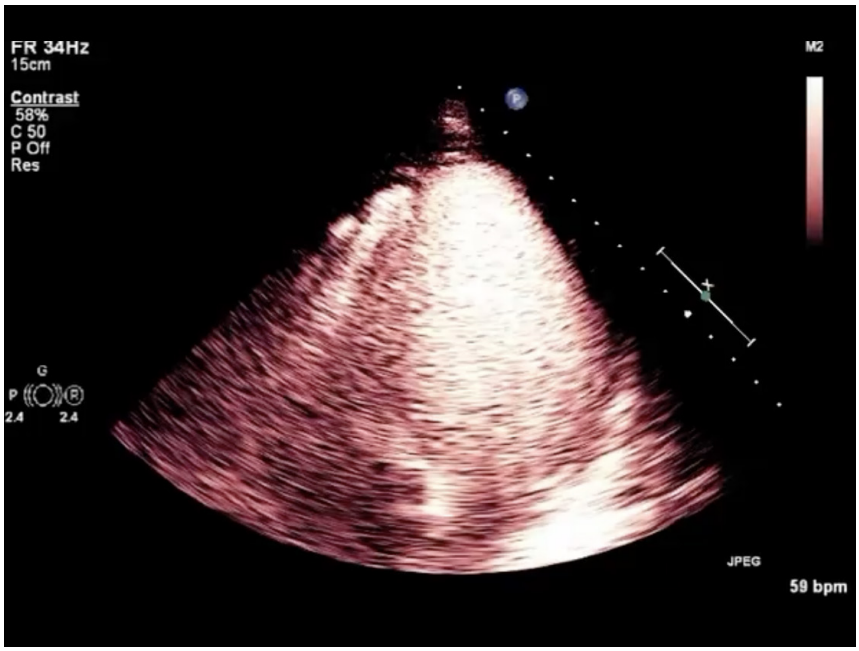
Supplementary Video 2 – *Transthoracic echocardiography – Apical two-chamber view. Complete opacification of the left ventricular cavity is observed, with an image suggestive of a thrombus in the apical region.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_02.mp4



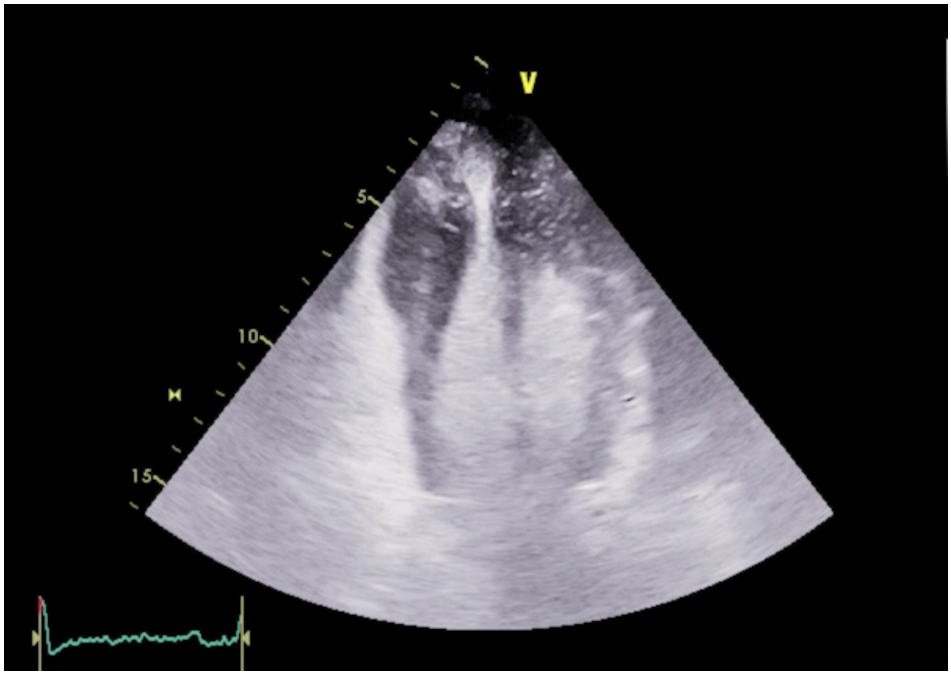
Supplementary Video 3 – *Transthoracic echocardiogram with real-time myocardial perfusion echocardiography technology, in the apical four-chamber view, showing a poorly perfused, homogeneous mass within the left atrium, characteristic of a left atrial myxoma.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_03.mp4



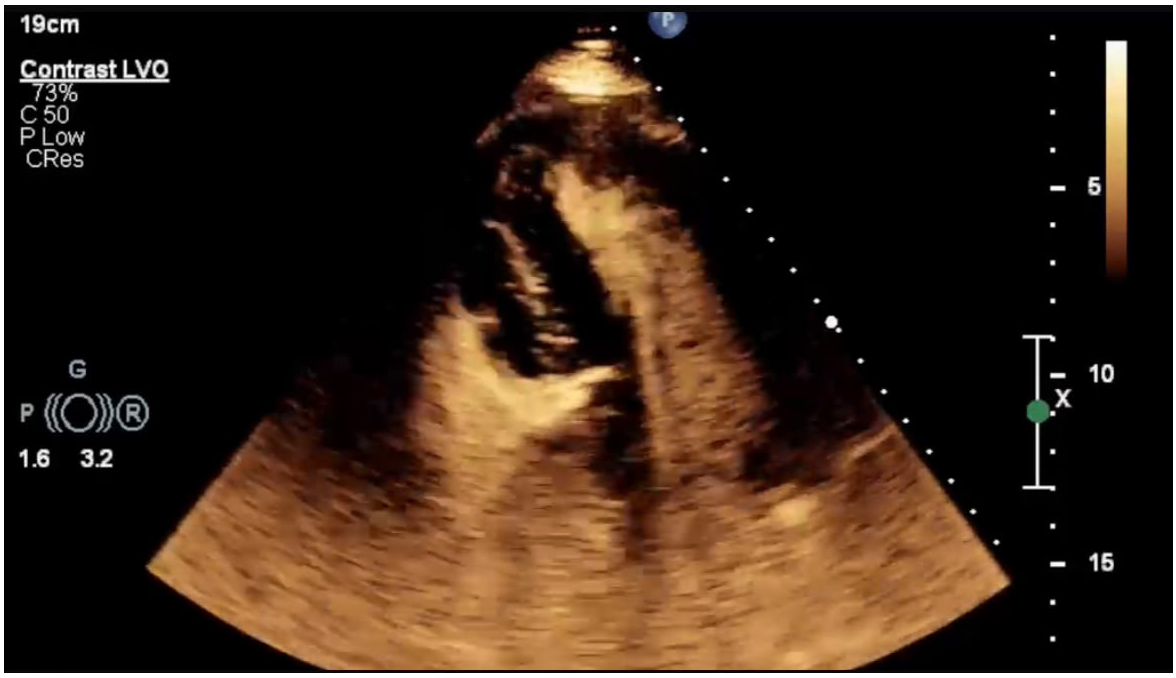
Supplementary Video 4 – *Transthoracic echocardiography – Apical four-chamber view without an ultrasound enhancing agent (UEA). The image shows a left ventricular (LV) apical aneurysm in a patient with a history of acute myocardial infarction 2 years earlier. The image also raises suspicion of an LV apical thrombus.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_04.mp4



Supplementary Video 5 – *Transthoracic echocardiography – Apical four-chamber view with an ultrasound enhancing agent (UEA). Following UEA injection and complete opacification of the left ventricle (LV), the apical LV wall-motion abnormality is confirmed, and the suspicion of an apical thrombus is ruled out.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_05.mp4



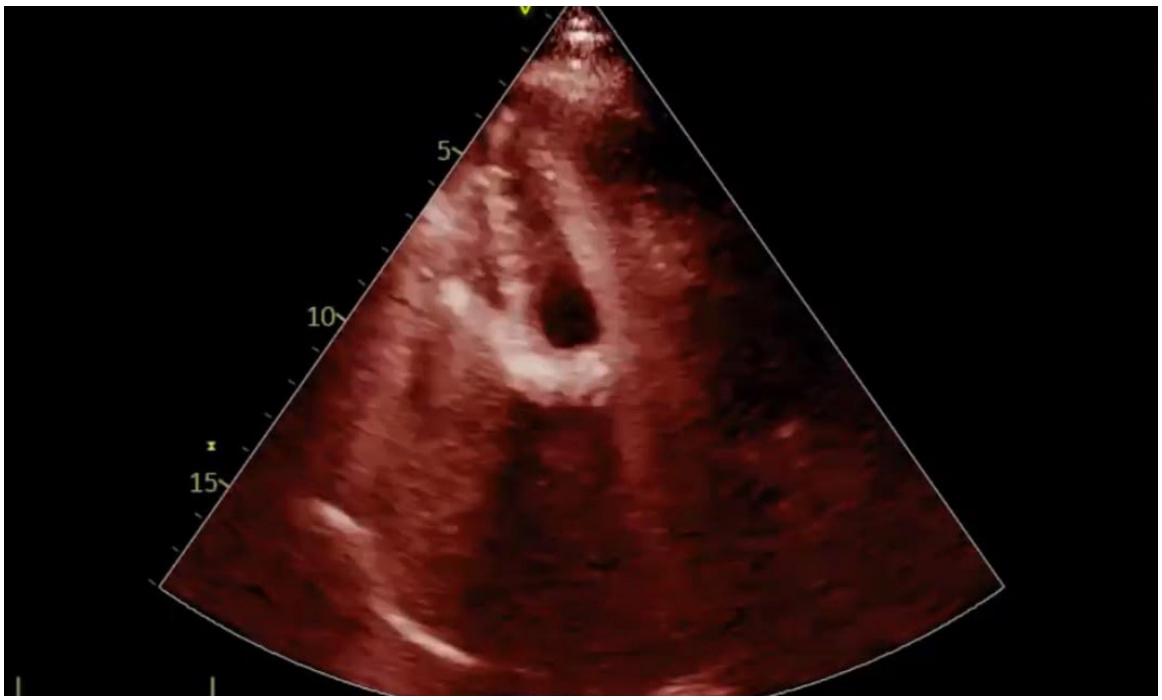
Supplementary Video 6 – *Midventricular hypertrophic cardiomyopathy with an apical aneurysm and no thrombus, clearly visualized with the aid of an ultrasound enhancing agent (UEA).*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_06.mp4



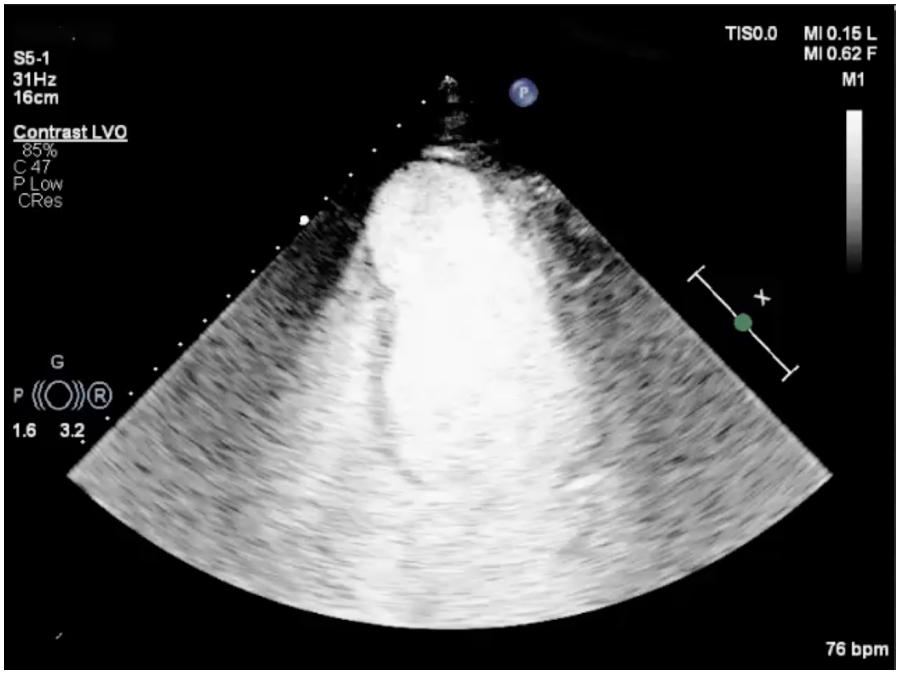
Supplementary Video 7 – *Use of UEA after first attempt at septal coronary artery selection, demonstrating perfusion of the mid-segment of the ventricular septum (topography unrelated to obstruction).*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_07.mp4



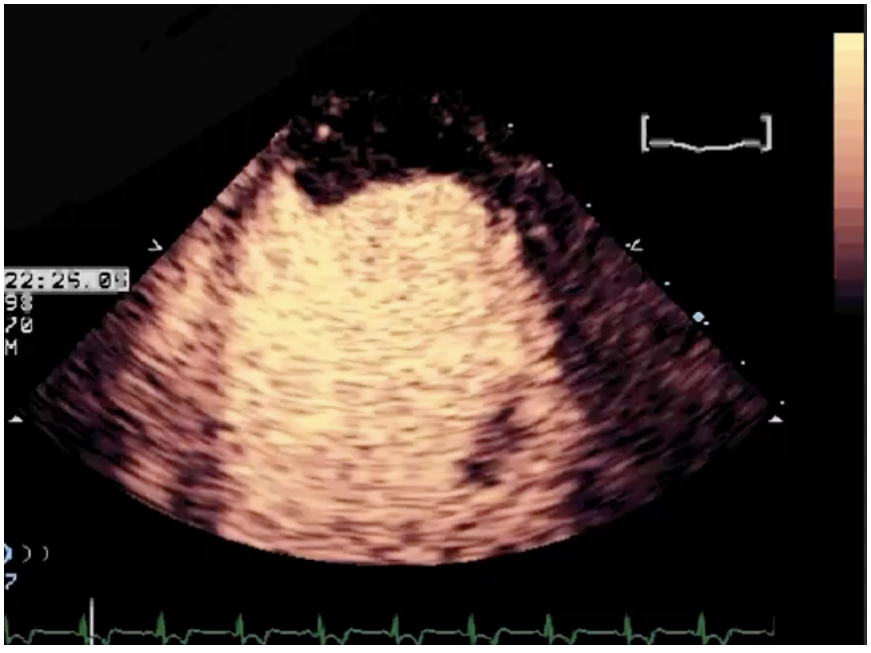
Supplementary Video 8 – *Use of UEA after adequate septal coronary artery selection, demonstrating perfusion of the basal segment of the ventricular septum (topography related to obstruction).*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_08.mp4



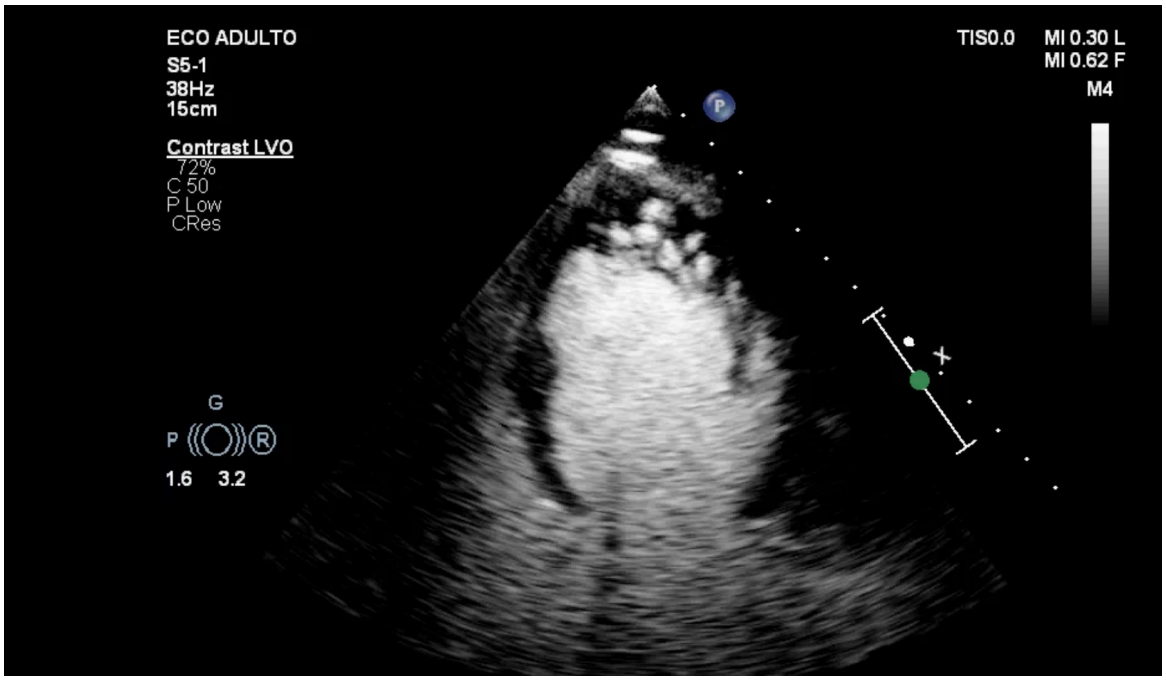
Supplementary Video 9 – *Use of UEA during alcohol septal ablation with evidence of right ventricular perfusion (moderator band).*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_09.mp4



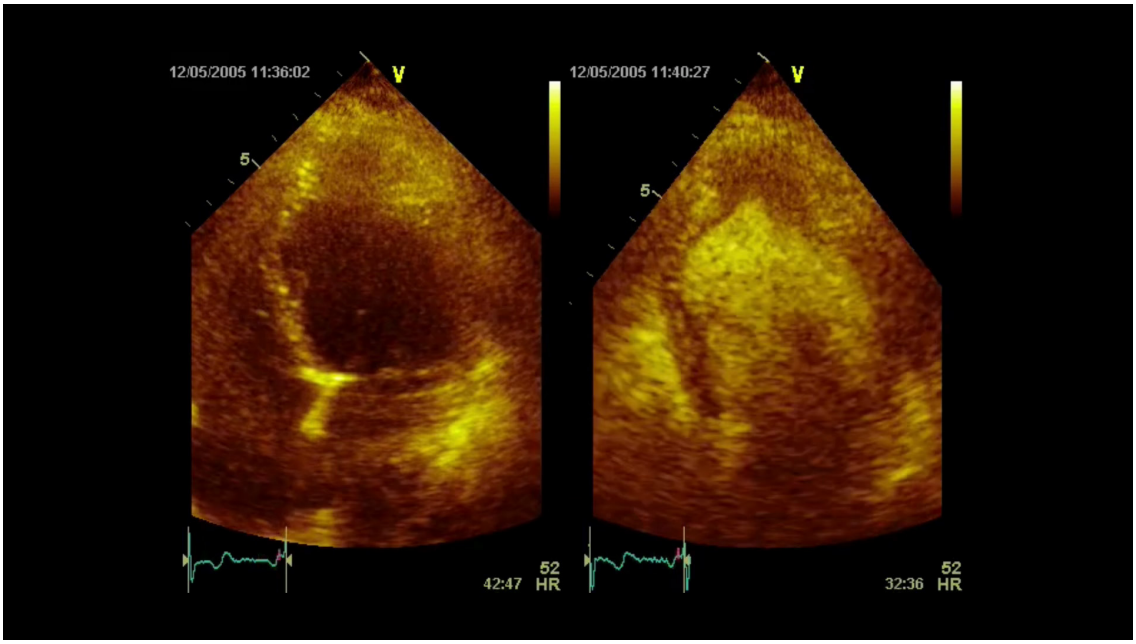
Supplementary Video 10 – *Transthoracic echocardiography – Apical four-chamber view. After complete opacification of the left ventricle (LV), an aneurysm of the apical region is observed, with no evidence of thrombus.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_10.mp4



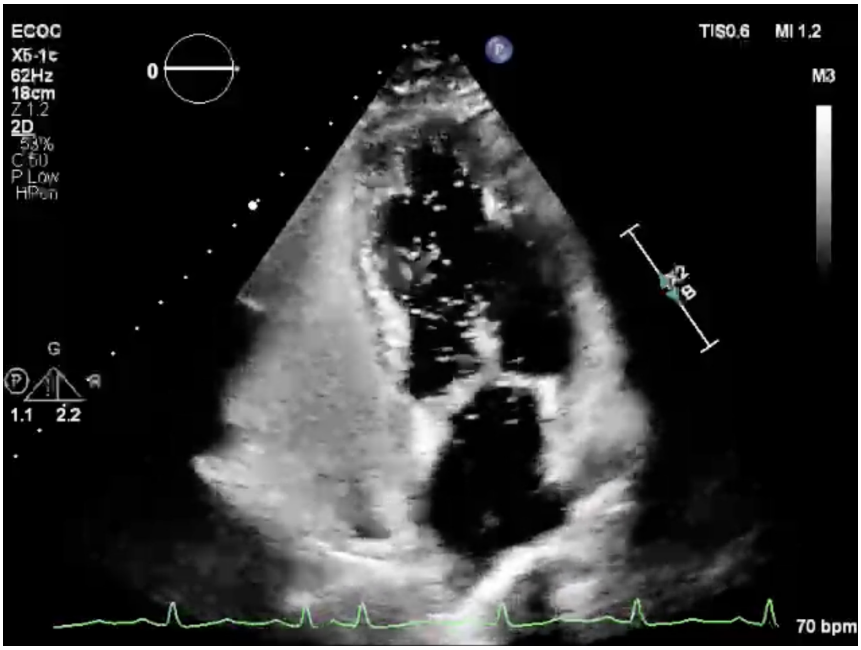
Supplementary Video 11 – *Transthoracic echocardiography – Apical four-chamber view with zoom of the left ventricle (LV). After complete LV opacification, akinesia of the apical region and a sessile mass related to the apical region are observed. The mass shows no enhancement with the ultrasound enhancing agent (UEA), consistent with thrombus.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_11.mp4



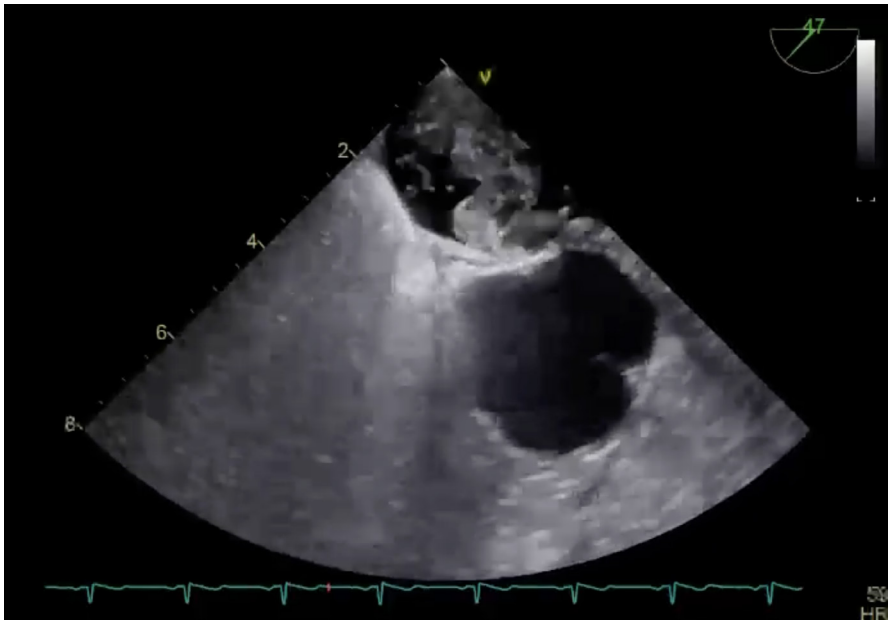
Supplementary Video 12 – *Transthoracic echocardiography – Apical four-chamber view. After complete opacification of the left ventricle (LV) with the ultrasound enhancing agent (UEA), hypertrabeculation of the mid and apical segments of the lateral wall is demonstrated.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_12.mp4



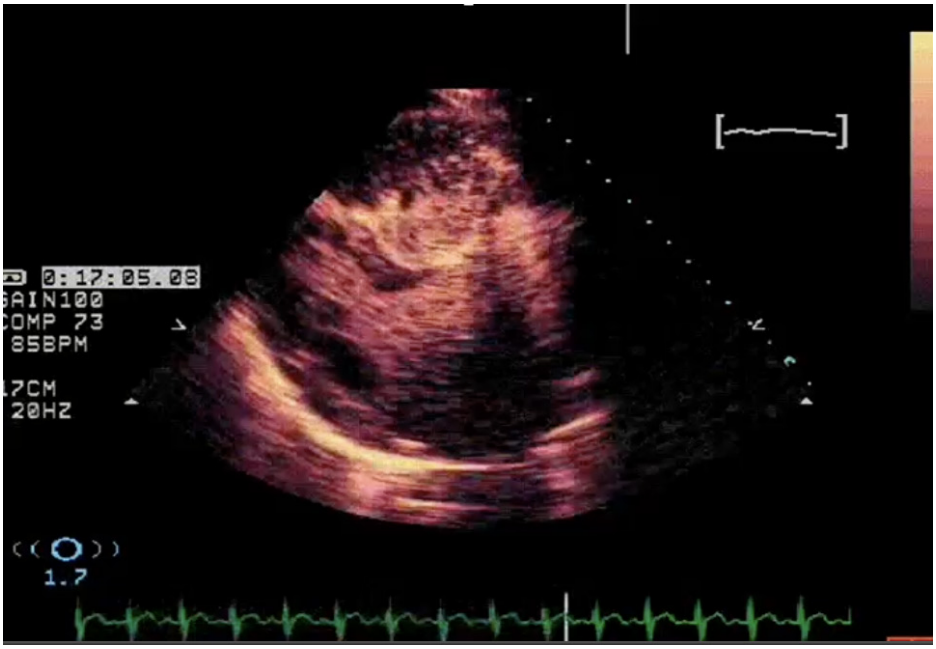
Supplementary Video 13 – *Transthoracic echocardiography – Apical four-chamber view with and without the use of an ultrasound enhancing agent (UEA). After complete opacification of the left ventricle (LV) with the UEA, marked increase in apical myocardial thickness is better visualized, a finding suggestive of hypertrophic cardiomyopathy, apical variant (Yamaguchi).*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_13.mp4



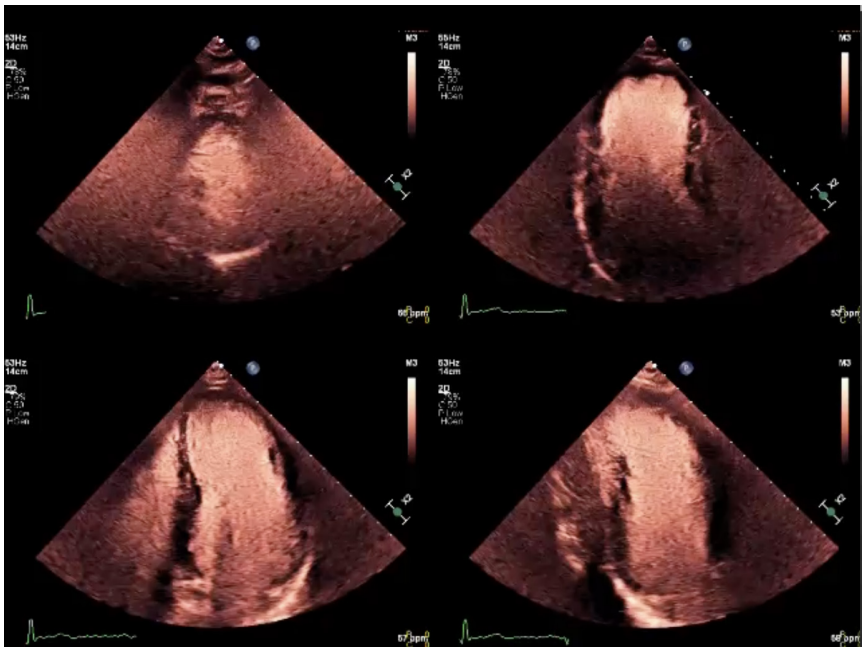
Supplementary Video 14 – *Transthoracic echocardiography – Apical four-chamber view. After peripheral intravenous injection of agitated saline and complete opacification of the right-sided cardiac chambers, early atrial transseptal passage of microbubbles into the left atrium is observed, consistent with a patent foramen ovale.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_14.mp4



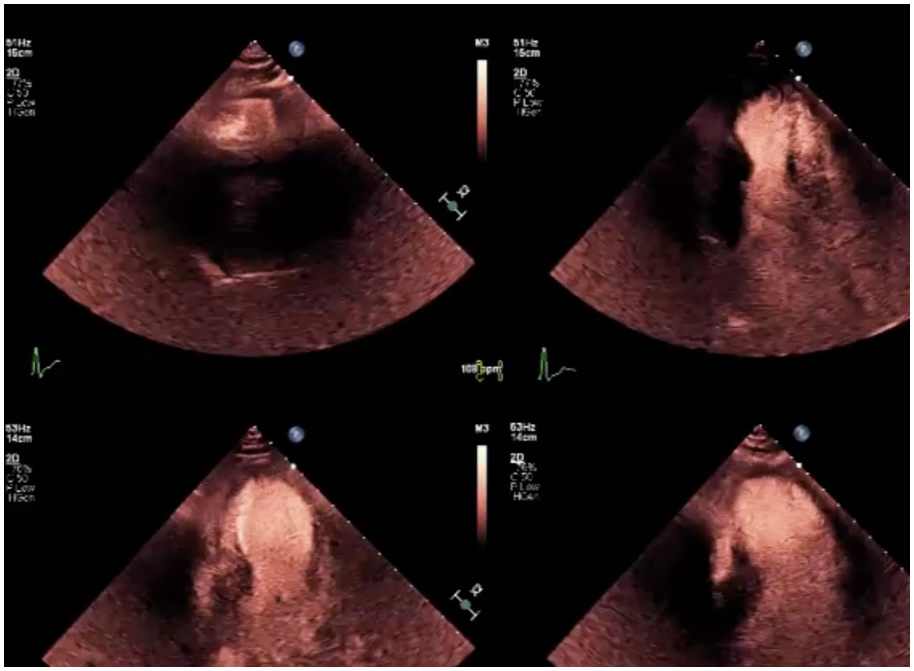
Supplementary Video 15 – *Transesophageal echocardiography – Mid-esophageal short-axis view at 45°, with zoom of the interatrial septum. The interatrial septum is redundant and exhibits increased mobility. After peripheral intravenous injection of agitated saline and complete opacification of the right atrium, separation of the fossa ovalis membrane and transseptal passage of microbubbles into the left atrium are observed, consistent with a patent foramen ovale.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_15.mp4



Supplementary Video 16 – *Transthoracic echocardiography – Parasternal long-axis view. An extensive, fixed mass with irregular borders and heterogeneous content is observed in the right ventricle. The mass shows areas of hyperenhancement with the ultrasound enhancing agent (UEA), suggesting hypervascularity, as well as nonenhancing regions that may correspond to areas of necrosis, findings suggestive of malignancy.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_16.mp4



Supplementary Video 17 – *Exercise stress echocardiography (Resting phase) – Parasternal short-axis and apical two-, three-, and four-chamber views. Complete opacification of the left ventricular cavity with the ultrasound enhancing agent (UEA) of all myocardial segments are observed, with no evidence of regional wall motion abnormalities.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_17.mp4



Supplementary Video 18 – *Exercise stress echocardiography (peak exercise) – Parasternal short-axis and apical two-, three-, and four-chamber views. Complete opacification of the left ventricular cavity with the ultrasound enhancing agent (UEA) and a hyperdynamic response of all myocardial segments are observed, with no evidence of regional wall motion abnormalities.*
Link: http://abccardiol.org/supplementary-material/2026/12310/realce_video_sup_18.mp4

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