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Review

Microbubble Enhanced Echocardiography in Current Cardiology Practice

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Abstract

Contrast-enhanced ultrasound imaging is a radiation-free clinical diagnostic tool that uses biocompatible contrast agents to enhance ultrasound signal, in order to improve image clarity and diagnostic performance. Ultrasound enhancing agents (UEA), which are usually gas microbubbles, are administered intravenously either by bolus injection or continuous infusion. UEA increase the accuracy and reliability of echocardiography, leading to changes in treatment, improving patient outcomes and lowering overall health care costs. In this review we describe: (1) the current clinical applications of ultrasound enhancing agents in echocardiography, with a brief review of the evidence underlying each of these applications; (2) emerging diagnostic and therapeutic applications of microbubble enhanced echocardiography (MEE), which rely either on the specific properties and composition of ultrasound enhancing agents or on the technical advances of clinical ultrasound systems; and (3) safety of MEE.

Keywords: microbubble enhanced echocardiography; ultrasound enhancing agents; contrast-enhanced ultrasound; contrast echocardiography; safety; review

1. Introduction

Echocardiography is the most commonly used imaging modality for assessing cardiac structure and function. However, an estimated 20% of echocardiographic studies may be suboptimal [1,2]. Hand-agitated saline resulting in rapidly dissolving air bubbles have been used for decades to enhance the ultrasound reflection from the right heart. The bubbles resulting from agitated solutions are too short-lived and too large to pass through the pulmonary bed, a property which has been used for the selective detection of right-to-left shunting (Fig. 1, Video 1) [3]. Suboptimal delineation of the left heart structures stimulated the development of commercial ultrasound enhancing agents (UEA) [4–6], specifically engineered so that the bubbles would be small enough (<8 μm) to pass through the capillaries in the lungs. Microbubble enhanced echocardiography (MEE) is now a well-established method, with a clinical history of more than 30 years. The technical details covering contrast infusion, and the specific transmit-receive and radiofrequency processing algorithms specifically designed to detect UEA microbubbles have been described and summarized by numerous other works [7]. In this review we will focus on the use of stable UEA designed to transit through the pulmonary circulation in clinical echocardiography. We will address both current and future promising diagnostic and therapeutic applications.

2. Methods

This narrative review considered landmark studies in the field of MEE, which were published between 1984 and 2021. Inclusion criteria were: studies with largely confirmed scientific impact, regardless of their size and design, which: (i) defined key technical and/or clinical aspects of the method; (ii) were very informative on specific clinical and/or technical applications; (iii) had been previously included in the European Association of Cardiovascular Imaging (EACVI) and American Society of Echocardiography (ASE) guidelines or (iv) were defined as landmark papers in the EACVI Key Reference Library (Contrast Echocardiography (https://www.escardio.org/), accessed on 15/03/2022). Only studies indexed in the PubMed database, and published in the English language were considered.

Ethics Approval and Consent to Participate

All patients included in this review as clinical illustrations gave their informed consent for the anonymous use of their clinical data and echocardiographic images.

3. Ultrasound Enhancing Agents

The current generation of UEA consist of microbubbles of comparable size to red blood cells (less than 8 μm in diameter), consisting of a gas encapsulated in a shell. The properties of these bubbles in the ultrasound field, and hence the image generated, are conditioned by their size, type of shell and gas. The interactions of the bubbles with

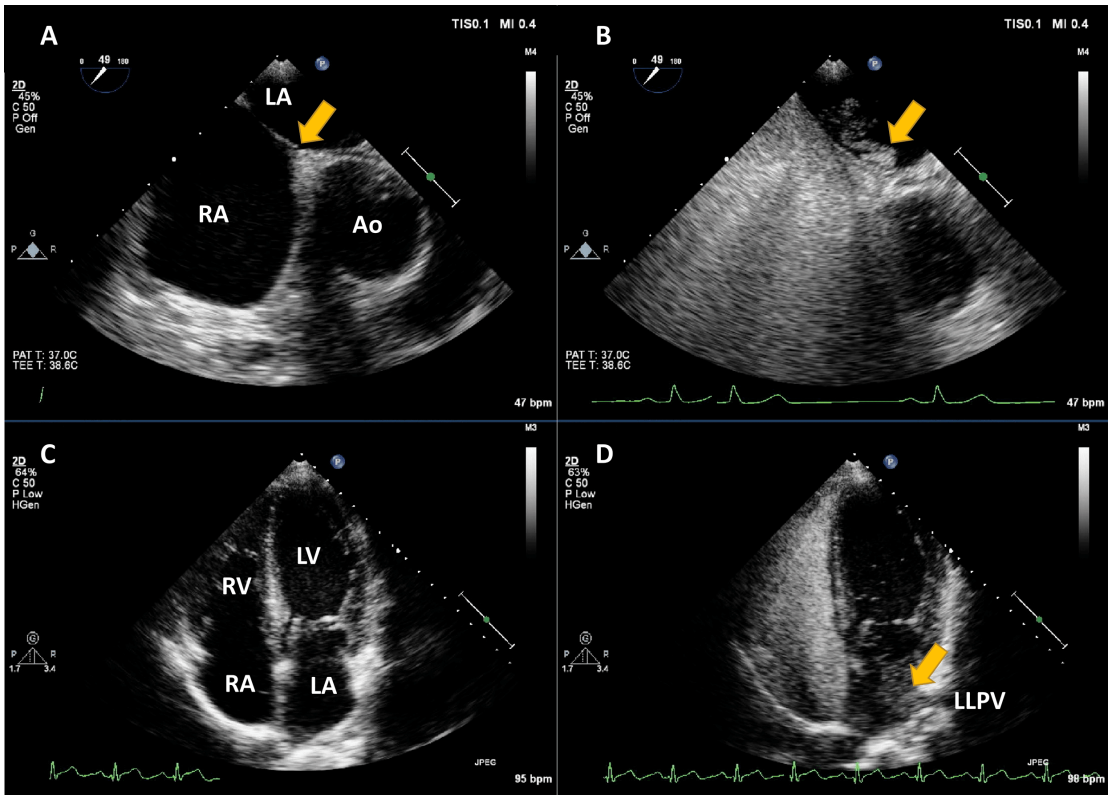
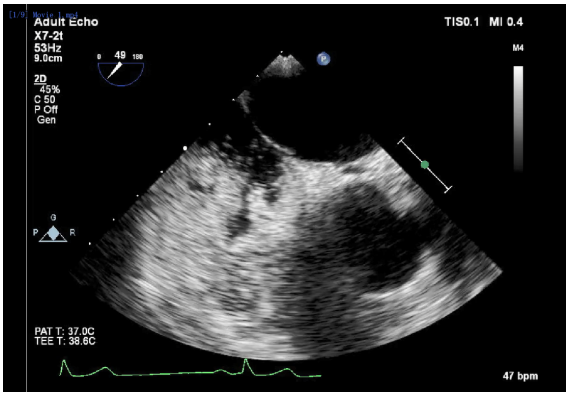


Fig. 1. Agitated saline injection for the detection of right-to-left shunts. (A,B) Example of intracardiac right-to-left shunting through a patent Foramen Ovale; transoesophageal echocardiography in mid-oesophageal position at 45°. The right atrium (RA), left atrium (LA) and interatrial septum at the level of the fossa ovalis are seen (arrow). After the infusion of agitated saline in a peripheral vein, a thick cloud of bubbles is seen passing through the patent *foramen ovale* (PFO) (arrow). (C,D) Right-to-left shunting through an intrapulmonary shunt, detected with transthoracic echocardiography, apical 4-chambers view. All four cavities are visualised. After the infusion of agitated saline in a peripheral vein, the right cavities are completely opacified. Seven heart cycles after the appearance of contrast in the right heart, a thick cloud of bubbles is seen in the LA (arrow), coming from the left lower pulmonary vein (LLPV). Source: personal collection.



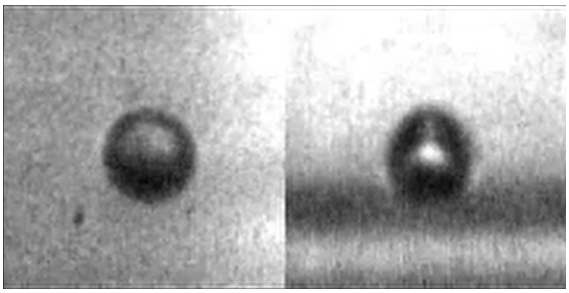
Video 1. Agitated saline injection for the detection of patent *foramen ovale*. (A,B) Transoesophageal echocardiography in mid-oesophageal position at 45°. The interatrial septum at the level of the fossa ovalis is seen. After the intravenous infusion of agitated saline, a thick cloud of bubbles is seen passing through the PFO. The movie corresponds to Fig. 1. The embedded movie may also be viewed at https://doi.org/10.31083/j.rcm2306202.

ultrasound are complex. Generally, these bubbles undergo compression and expansion when exposed to the ultrasound waves (Video 2). These interactions can be generally divided in stable cavitation (oscillation of the bubbles without destruction) or inertial cavitation (abrupt oscillation disrupting the shell). These oscillations produce scattering (i.e., linear signal), which is proportional with the bubble size. However, one of the most important properties of microbubble oscillation is that they produce non-linear signal [8], which can be detected by the ultrasound system at harmonic frequencies.

The microbubbles must be strong enough to resist significant destruction with the clinical ultrasound incident power output. This has been achieved by using lipid or albumin shells, encapsulating inert high-molecular weight gas cores [7,9] (Table 1).

Table 1. Current commercial ultrasound enhancing agents.

Agent	Manufacturer	Shell	Gas	Average size (μm)
Optison	GE Healthcare	Albumin	Perfluoropropane	2–4.5
Definity/Lumify	Lantheus Medical Imaging	Lipid	Perfluoropropane	1.1–3.3
Sonovue/Lumason	Bracco Diagnostics	Amphiphilic phospholipid	Sulphur hexafluoride	2–3



Video 2. Ultrasound enhancing agent microbubble oscillation induced by an ultrasound pulse. Images were obtained with a Brandaris-128 ultra-fast framing camera at a frame rate of 15.3 Mfps. (movie courtesy of Dr HJ Vos). The embedded movie may also be viewed at https://doi.org/10.31083/j.rcm2306202.

4. Clinical Applications

4.1 Left Ventricular Opacification (LVO)

The largest body of evidence for MEE concerns the indication for LVO for enhancing the endocardial borders [10–15]. This is achieved by using repetitive intravenous boluses of UEA, and sometimes continuous low-dose infusion. Guidelines indicate the use of LVO to enhance the endocardial borders in cases when the LV dimensions, function or regional wall motion cannot be accurately assessed using non-enhanced ultrasound [7,14]. The general "rule of thumb" is to use microbubble UEA in cases where two or more contiguous myocardial segments are not properly visualized with non-enhanced ultrasound [13]. Of course, recent years have seen tremendous improvement in image quality for clinical ultrasound systems. But despite the introduction of harmonic imaging as a standard, some images remain non-diagnostic (Fig. 2). Moreover, harmonic imaging represented a significant leap in MEE [16,17], leading to the present-day contrast-specific imaging modalities.

4.1.1 LV Size and Ejection Fraction (EF)

By using UEA, enhanced echocardiographic measurements of LV volumes and ejection fraction are very close to the reference cardiac magnetic resonance (CMR) values [11,12], and significantly less variable as compared to unenhanced imaging, even if baseline images are of good quality [18]. This significant difference in quality, information and accuracy leads to a clinical impact on diagnosis and management [6,19,20]. Echocardiographic estimates of LV volume tend to be larger when using LVO, mainly because it aids the exclusion of trabeculae (Fig. 3), making the measurements closer to their CMR counterparts [13].

3D MEE is not yet a standard. Although one multicenter study demonstrated that it may improve inter-observer variability and accuracy [11,12], there are still limitations because of UEA destruction in the near field generated with 3D echo, which in other studies resulted in increased inter-observer variability [21].

As such, LV microbubble-enhanced echocardiographic volumes are not presented in the current guidelines for chamber quantification [22], and they should not be compared with the non-enhanced values mentioned in these guidelines. New reference ranges for LV enhanced echocardiographic volumes should be established by large studies.

The evidence regarding the use of MEE for linear size measurements of the LV is less strong. Recent monocentric studies suggest a benefit of measuring the interventricular septal size in hypertrophic cardiomyopathy with MEE, and the results may be closer to the reference CMR and smaller than non-enhanced values [23,24]. However, precise reference ranges for MEE LV size and wall thickness in parasternal views are yet lacking, and CMR should be preferred whenever possible in the workup of HCM patients. Also, for the posterior wall thickness in the parasternal views MEE may surprisingly not be better than non-enhanced images [24], and this because the significant signal attenuation through interposition of the microbubbles in the right and left ventricular cavities. This is even more obvious at higher concentrations of UEA.

4.1.2 Assessment of Regional Wall Motion at Rest

The significant improvement in endocardial delineation demonstrated a similarly significant benefit in the analysis of regional wall motion abnormalities [25]. When analyzed by a panel of experts, the accuracy in diagnosing wall motion abnormalities was highest for CMR (84%), followed by 2D MEE (78%) and 3D contrast (76%) [25]. In addition to this multicenter study, other works demonstrated the benefit of contrast imaging in the interpretation of LV wall motion in intensive care patients [26], or post myocardial infarction [19,27]. The benefit becomes more obvious when used as point-of-care echocardiography, to rapidly identify wall motion abnormalities in patients with suspected coronary artery disease (Video 3).

4.1.3 Assessment of LV Wall Structure

Another application of LVO is morphological diagnosis, particularly in disease states which manifest in the artefact-prone LV apex. Beside possible foreshortening,

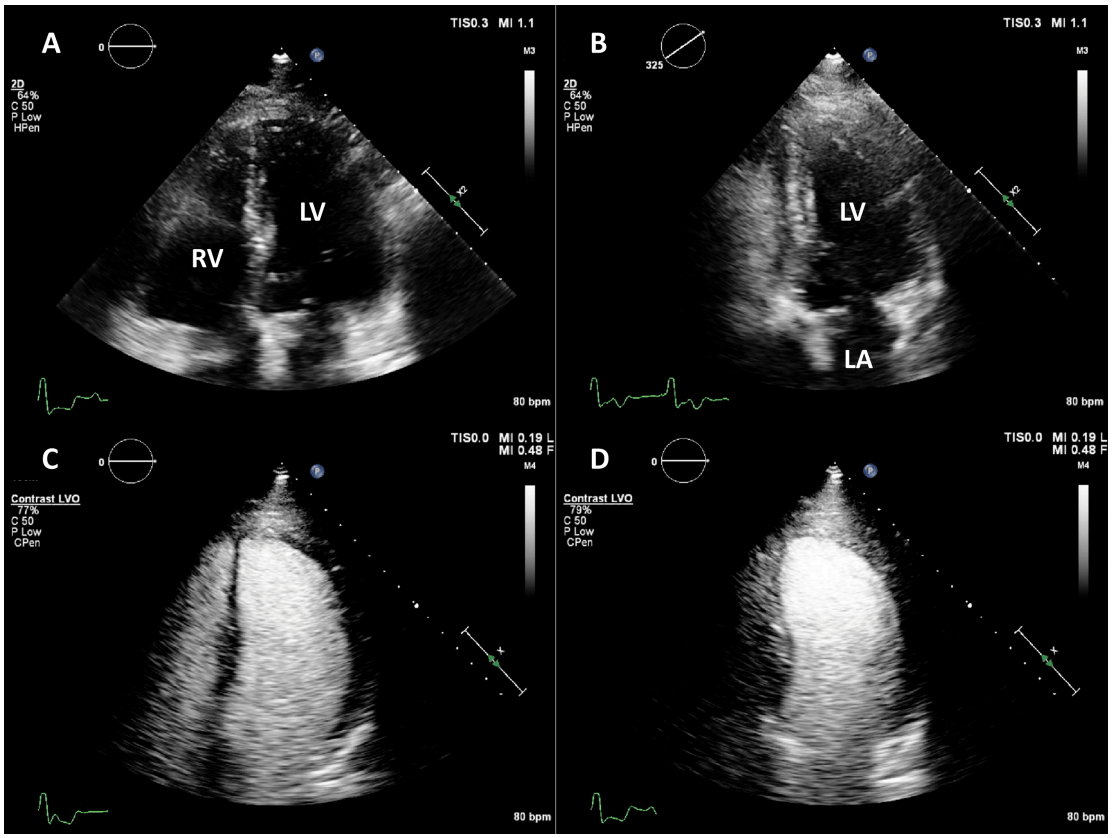


Fig. 2. Left ventricular opacification (LVO) for endocardial border delineation. Example of baseline non-enhanced echocardiography images in apical 4-chambers (A) and apical 2-chambers (B) views, where the visualization of the endocardium is suboptimal over several segments. After intravenous injection of a bolus of UEA, there is full opacification of the LV cavity, with clear delineation of the endocardium in all segments (C, D). Source: personal collection.

the LV apex is prone to clutter and reverberation artefacts, while also having a weaker potential to generate harmonics because of its position in the near-field in apical views [28]. As such, apical forms of hypertrophic cardiomyopathy [29,30], eosinophilic cardiomyopathy [31] and non-compaction cardiomyopathy [32] may escape detection with unenhanced ultrasound.

Numerous case reports and case series document the use of UEA in these instances [29–33]. Studies have also been performed demonstrating the added value of contrast-enhanced ultrasound in hypertrophic cardiomyopathy [34] (Fig. 4, Video 4).

4.1.4 Left Atrial Appendage Visualization during Transoesophageal Echocardiography

Transoesophageal echocardiography is an established method for assessing the left atrial appendage (LAA) for the presence of thrombi [35,36] or to guide LAA interventions (Fig. 5, Ref. [36]). In case of LAA stasis, the dense spontaneous contrast may mask the presence of a small thrombus. Several studies demonstrated that the adjunction of UEA increases the diagnostic yield of the procedure [37,38] and reduces subsequent strokes [39].

However, contrast-specific transoesophageal applications are not available on all ultrasound clinical systems, therefore in such cases non-contrast mode harmonic 2D imaging may be used, with a mechanical index (MI) under 0.3 (Fig. 6, Video 5).

The significant improvement LVO provides in accuracy, reproducibility and confidence in the assessment of LV size, shape and function encourages the incorporation of this relatively low-cost method in the standard exploration of clinical patients. This has been recognized by the current guidelines [7,13,22]. It seems reasonable to systematically use UEA for LVO in patients in whom two or more segments are not adequately visualized with non-enhanced ultrasound.

4.2 Stress MEE (LVO)

The addition of UEA during stress echocardiography protocols is usually achieved through an LVO application with low-MI harmonic imaging (Fig. 7, Video 6). The result is an increase in the likelihood of a diagnostic test, a better visualization of all myocardial segments, study quality and reader confidence, as compared to invasive or non-invasive reference [40–43]. The addition of UEA to non-enhanced studies resulted in a better agreement with coronary angiography, even in patients with intermediate coro-

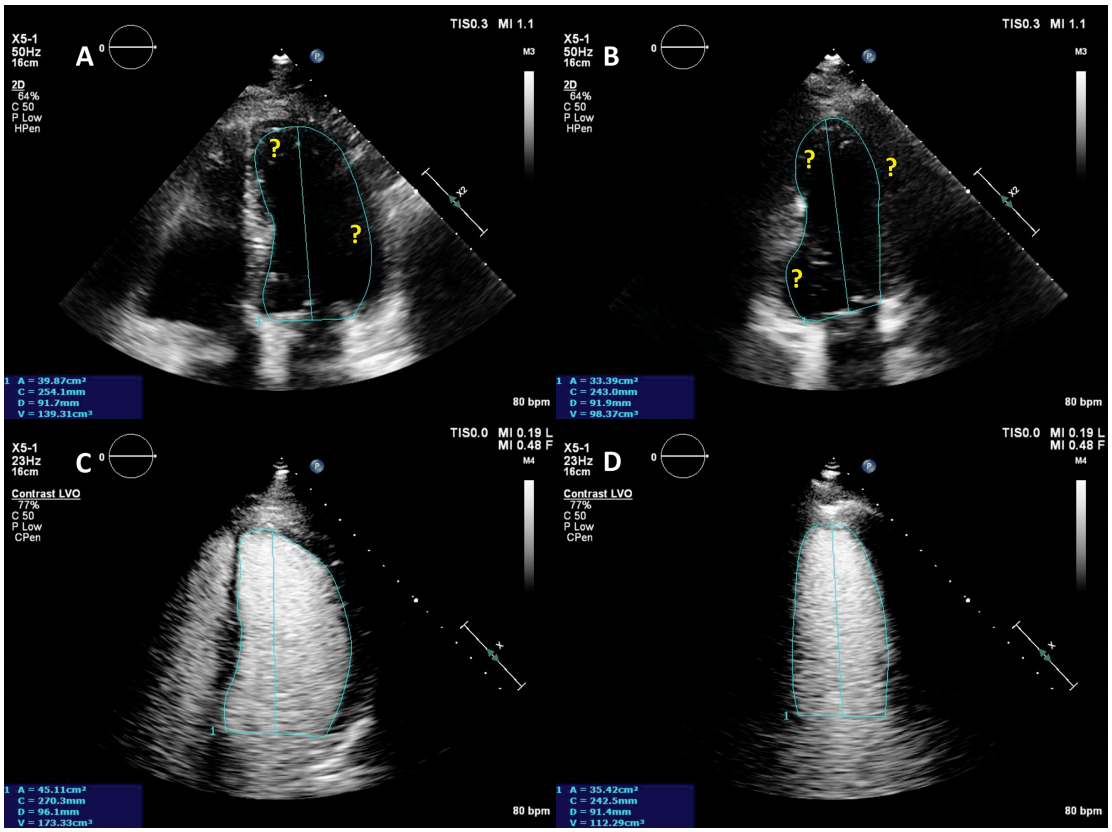
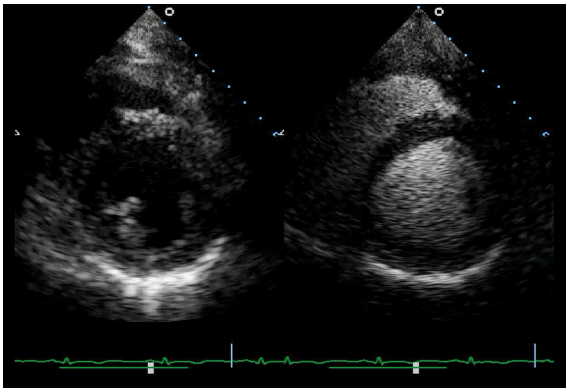


Fig. 3. Ejection fraction (EF) estimation. (A,B) Non-enhanced ultrasound images. The endocardium is not clearly visible in several segments (question marks), making the volumes difficult to assess. (C,D) Contrast-enhanced images. The endocardial border is clearly defined, allowing for a biplane volume estimation. Moreover, the LV end-diastolic volumes in contrast-enhanced images is notably larger than the one on the non-enhanced images, probably because of a combination of insufficient image quality on native images, and exclusion of trabeculae and papillary muscles on contrast images. Source: personal collection.



Video 3. Patients with non-specific thoracic pain and non-diagnostic electrocardiogram. Non-enhanced images (left) in the parasternal short axis of the left ventricle at the level of the papillary muscles lack definition in the interventricular septum, no clear motion abnormality is seen. With contrast (right) the endocardium is clearly seen and hypokinesia is noted in the interventricular septum. The embedded movie may also be viewed at https://doi.org/10.31083/j.rcm2306202.

nary lesions [44]. Of course, the use of LVO in stress echo has the largest impact in patients with suboptimal image [45]. Nevertheless, contrast-enhanced ultrasound also improves the wall motion score and detection of regional wall motion abnormalities in patients with adequate image quality [46]. Contrast-enhanced dobutamine stress echocardiography provided adequate risk stratification in patients with increased cardiovascular risk due to obesity or suspected coronary artery disease [47–49].

In patients with incomplete visualization of at least 2 contiguous segments contrast should be used for stress echocardiography. In patients with adequate image quality, contrast could be used to assess the myocardial perfusion, in addition to wall motion [7,13].

4.3 Myocardial Perfusion Imaging

4.3.1 Detection of Coronary Artery Disease

UEA have an intravascular behavior which mimics closely that of red blood cells [50], and an in vivo distribution to the intravascular compartment, making them ideal for assessing microvascular distribution in the tissue. The total blood volume in the coronary circulation at rest is around 12 mL/100 g of myocardial tissue [51] and 90% of

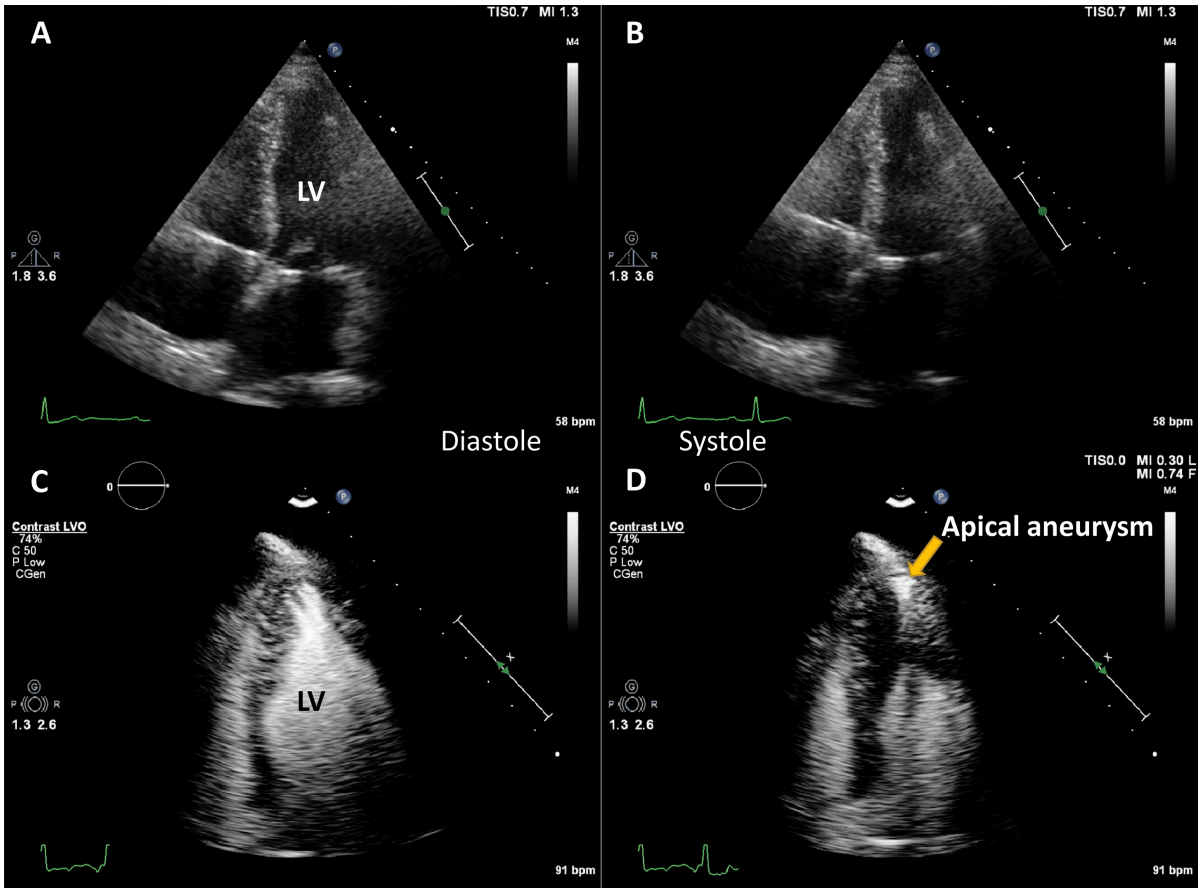
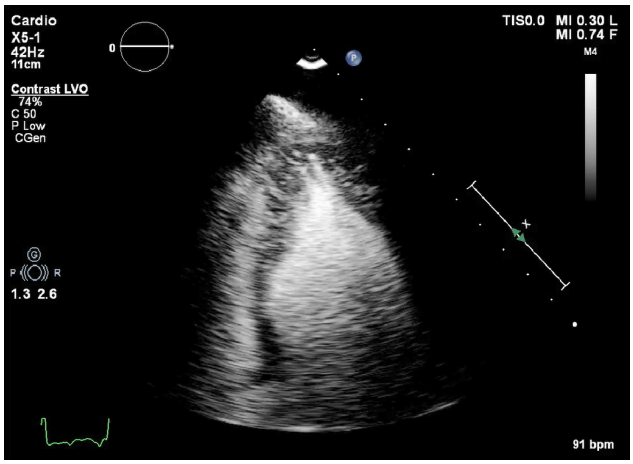


Fig. 4. Hypertrophic cardiomyopathy patient with very poor image in apical 4-chambers view. (A,B) Native images, end-diastole (A) and end-systole (B). The endocardium of the lateral wall is not visible, and the apex cannot be seen. (C,D) Contrast-enhanced images, in the same moments in the cardiac cycle. The LV contour is clearly delineated, during systole there is complete cavity obliteration, with an apical aneurysm (arrow). Source: personal collection.



Video 4. Apical hypertrophic cardiomyopathy, microbubble-enhanced echocardiography. The images correspond to the patient in Fig. 4. There is mid-cavity obliteration, with a dyskinetic apical pouch (apical aneurysm), which were not seen on native images. The embedded movie may also be viewed at https://doi.org/10.31083/j.rcm2306202.

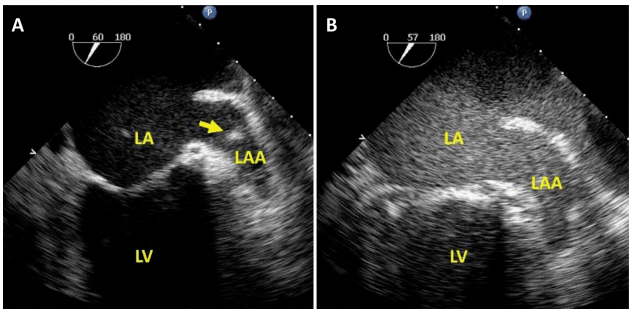


Fig. 5. Ultrasound-enhanced TEE used to facilitate the safety of an atrial fibrillation cardioversion procedure. (A) Mid-esophageal view at 60°, demonstrating a hyperechoic signal (arrow) inside the left atrial appendage (LAA), which could be an artefact, but a thrombus cannot be excluded because of "smoke" in the LAA. (B) By adding intravenous ultrasound enhancing agent, the LAA appears free of any abnormal echo. In the absence of thrombus, the cardioversion was successfully performed. Modified with permission from Doukky R *et al.* [36].

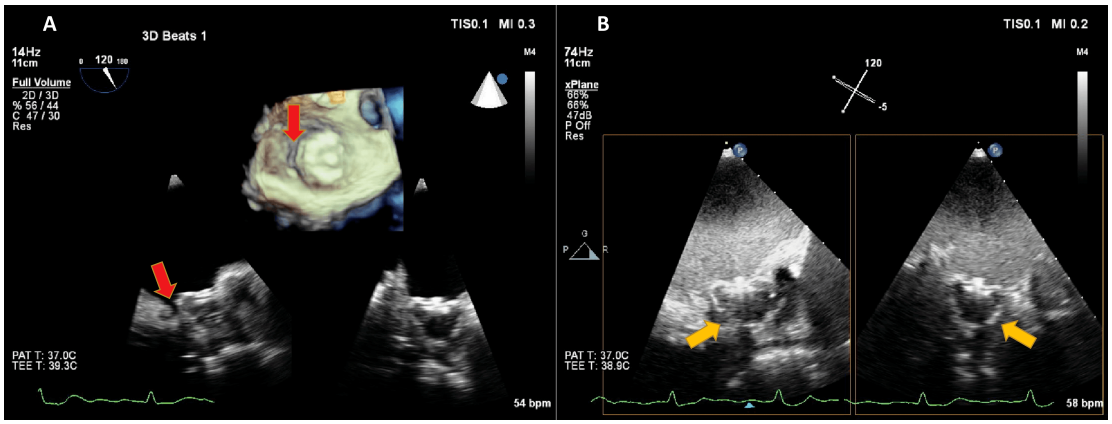
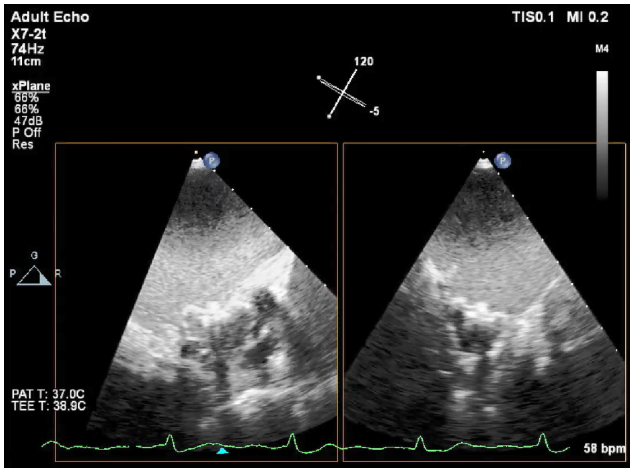


Fig. 6. Transoesophageal echocardiography in a patient with a Watchman left atrial appendage (LAA) closure device. Microbubble-enhanced ultrasound was used in order to detect a suspected residual leak around the occluder. On non-enhanced multiplane and live 3D images (A) a gap is visible (red arrows) between the rim of the device and the LAA. Because of shadowing, the sealing of the LAA cannot be verified. By adding intravenous contrast (B) and using harmonic imaging with a mechanical index of 0.2, contrast is seen in the LAA, surrounding the device (yellow arrows), demonstrating incomplete sealing of the LAA. Source: personal collection.



Video 5. Microbubble-enhanced transoesophageal echocardiography biplane images focused on the left atrial appendage (LAA). In the LAA a closure device (Watchman) is present, but the sealing is incomplete, contrast can be seen all around the device, enhancing its borders. The patient corresponds to Fig. 6. The embedded movie may also be viewed at https://doi.org/10.31083/j.rcm2306202.

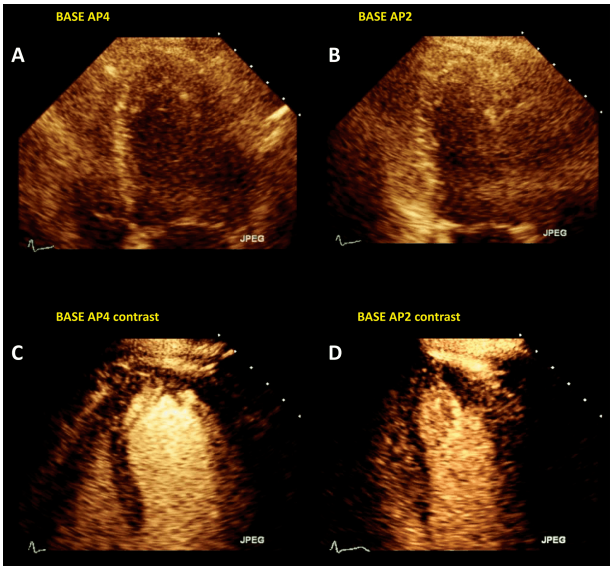
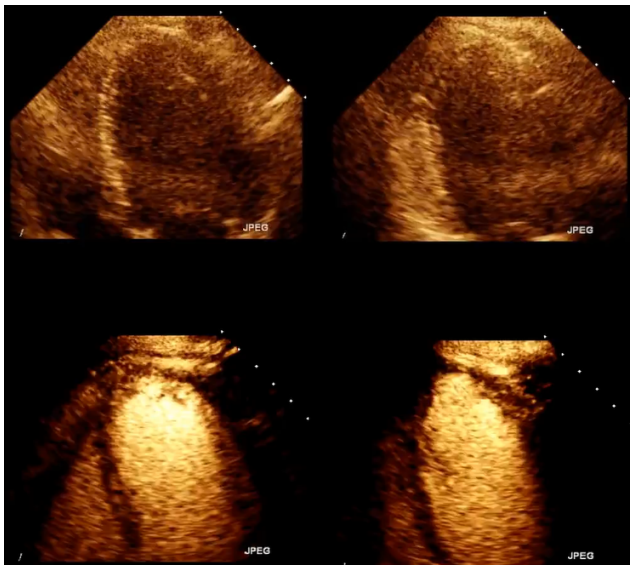


Fig. 7. Microbubble enhanced stress echocardiography. Baseline non-enhanced images are recorded in apical 4 and 2 chambers (A,B), demonstrating insufficient delineation of the endocardial borders; With contrast (C,D) the LV contours become clearly visible. Source: personal collection.

this volume is found in the capillaries. If the myocardium is fully saturated during a continuous UEA infusion, the UEA in the myocardium will reflect the distribution of the capillary circulation [52]. This means that simply measuring the intensity of signal enhancement with UEA provides a quantitative measure of intact microcirculation in the myocardium. Using this approach, the extent and distribution of viable (perfused) myocardium can be assessed (Fig. 8, Video 7) with a sensitivity and specificity similar to single photon emission computed tomography (SPECT) techniques [41] or magnetic resonance imaging (MRI) [53].

Experimental data demonstrated that the myocardial capillary flow has a velocity of 1 mm/s within a beam elevation of 5 mm. This means that the total filling of the capillaries takes around 5 seconds (5 cardiac cycles at a heart rate of 60/min) at rest [54]. If for some reason the blood flow is slowed down (stenosis) or accelerated (vasodilation), this time will be modified accordingly [54]. During stress the myocardial blood flow increases 4–5-fold, which means that replenishment will be achieved in 1 to 2 seconds



Video 6. Dobutamine stress echocardiography, baseline images. The upper panels are non-enhanced, LV walls and endocardial borders are difficult to see. The lower panels show the contrast-enhanced images, the LV contours and wall motion are clearly visible. The movie corresponds to Fig. 7. The embedded movie may also be viewed at https://doi.org/10.31083/j.rcm2306202.

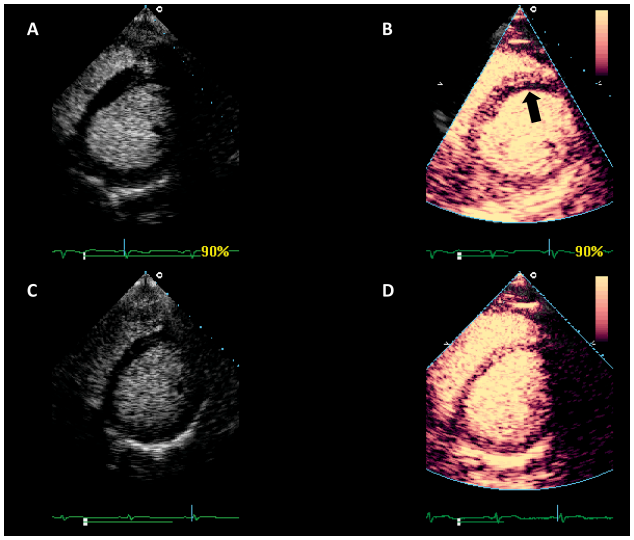
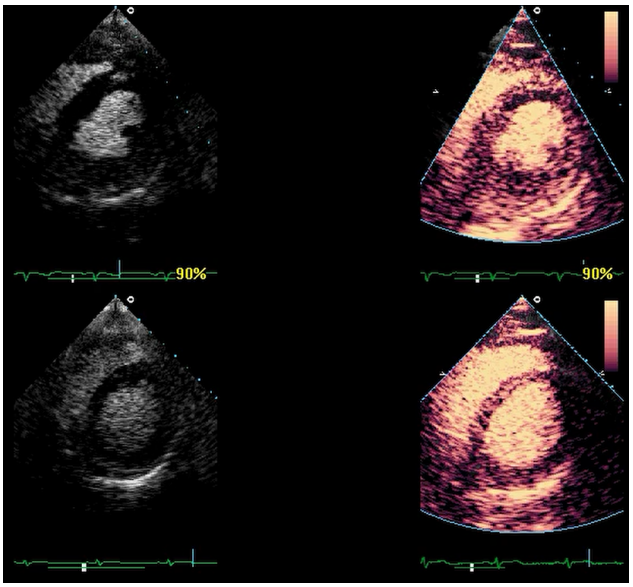


Fig. 8. Detection of coronary artery disease by using myocardial perfusion imaging. Patient with dyspnoea but no typical chest pain. (A) LVO images demonstrate a discrete anomaly in the septal kinetics. (B) Myocardial perfusion imaging shows a subendocardial perfusion defect in the anterior and anteroseptal segments. Patient underwent coronary angiography and stent placing in the left anterior descendent coronary. (C) Follow-up study before discharge: LVO normal kinetics. (D) Follow-up perfusion study on discharge: homogeneous perfusion. Source: personal collection.



Video 7. Detection of coronary artery disease by using myocardial perfusion imaging. Upper panels: acute phase; Lower panels: discharge. In the acute phase myocardial perfusion imaging (upper right panel) shows a subendocardial perfusion defect in the anterior and anteroseptal segments, with mild hypokinesia. Patient underwent coronary angiography and stent placing in the left anterior descendent coronary. Perfusion was homogeneous at discharge. The movie corresponds to Fig. 8. The embedded movie may also be viewed at https://doi.org/10.31083/j.rcm2306202.

(2–3 cardiac cycles at a heart rate above 120/min).

Assessing myocardial perfusion has incremental benefit over wall motion analysis in detecting coronary artery disease (CAD) [55]. The myocardial contrast signal obtained in a steady state (continuous infusion) can be normalized to the LV cavity signal and this represents the myocardial capillary volume [54]. By delivering a series of high-power (high mechanical index) ultrasound frames to the region of interest, cavitation and destruction of the UEA bubbles is initiated; the analysis of the progressive recovery of the contrast signal in the myocardium provides information on the myocardial capillary flow [54–56] (Fig. 9, Video 8).

The sensitivity and specificity of myocardial contrast stress echocardiography in detecting CAD are 83% and 79% respectively for a vasodilator stress (dipyridamole or adenosine) and 88% and 77% respectively for dobutamine or exercise stress studies [7,57]. Two large multicenter studies demonstrated superior sensitivity of myocardial perfusion stress echocardiography as compared to SPECT, but lower specificity [58,59]. The higher sensitivity may be due to the fact that SPECT only detects the myocardial blood volume, and not the kinetics of myocardial blood flow, as opposed to contrast-enhanced stress echocardiography, which can assess both [54]. The lower specificity may be related to artefacts during stress echocardiography, mainly

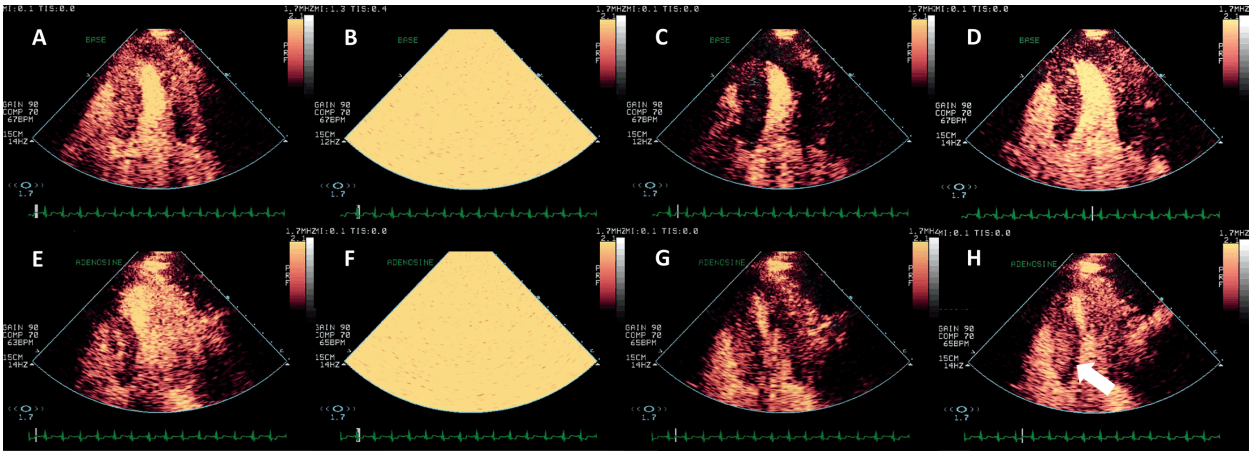
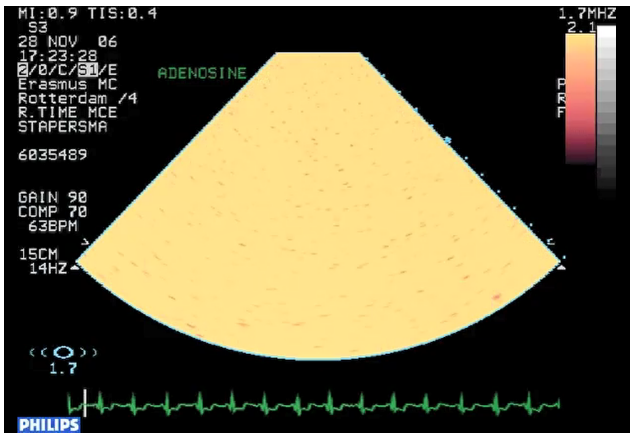


Fig. 9. Vasodilator stress echocardiography with Adenosine, using a low-MI setting and flash-replenishment technique. (A–D) Baseline end-systolic frames before flash (A), flash (B), immediately post flash (C, myocardium is dark because of the destruction of contrast), and post-replenishment frame (D) when perfusion is again homogeneous. (E–H) After adenosine infusion, the same order of frames. (H) Post replenishment end-systolic frame demonstrating a subendocardial perfusion defect in the inferosetptal segements (arrow). Of note, during stress myocardial replenishment occurs faster than at rest because of the pharmacological vasodilation. The patient underwent coronary angiography and stent placing in the right coronary artery. Source: personal collection.



Video 8. Adenosine contrast-enhanced stress echocardiography, with a flash-replenishment cycle. After the flash there is a persistent perfusion defect in the inferoseptal segments. The movie corresponds to Fig. 9. The embedded movie may also be viewed at https://doi.org/10.31083/j.rcm2306202.

in the apex (near-field destruction) and basal segments (far field attenuation in apical view).

There is now a large body of evidence supporting the added value of myocardial perfusion imaging over wall motion assessment alone in stress echocardiography [55,60–66]. When using vasodilator stress, the use of high-power flash-replenishment technique is likely more important than during dobutamine or exercise stress, where wall motion abnormalities and perfusion defects may be more evident because of the higher oxygen demand during this type of stress [67].

4.3.2 Myocardial Viability

As mentioned earlier, the intramyocardial UEA signal intensity correlates with the microvascular density in the area, and would naturally be lower in regions with high collagen content [68,69]. Dobutamine stress echocardiography is routinely used for the assessment of myocardial viability. A contractile response during stress relates to the presence of the microvasculature and the presence of a blood flow reserve, both of which are particularly well predicted by MEE. An increasing body of evidence suggests that MEE is a useful and highly feasible technique for the evaluation of myocardial viability [53,70–72].

4.3.3 Coronary Flow Reserve

Quantitative approaches to evaluate myocardial perfusion or myocardial flow rate (the β-value = the rate at which the myocardial blood volume transits the tissue [54]) have been shown to correlate well with coronary flow, fractional flow reserve and positron emission tomography [41,54,73–75]. This can be achieved both with high as well as with low MI applications. For the high-MI the myocardium is first cleared of contrast (flash) and then replenishment is assessed with triggered high-MI imaging or continuous low-MI. The blood flow is estimated as the product of peak acoustical intensity (db) and flow velocity (db/s), and compared with the values obtained during stress, leading to a good correlation with invasive coronary flow reserve [73].

It is essential that in quantitative methods, relative indices are used, such as the ratio of stress and rest blood flow. The interaction between ultrasound power and microbubble concentration is variable in each patient and with each ultrasound system, which affects the absolute values of myocardial blood volume and blood flow velocity.

4.4 Intracardiac Masses

UEA have largely been used in the detection of LV thrombi [76–78]. This is in fact an application of the LVO method, enhancing the delineation between the wall, cavity and the thrombus mass (Fig. 10, Ref. [79]). However, prognostic implications for small mural thrombi are not clear. Myocardial perfusion detection, as described above, may help to diagnose small intracardiac or even intramural masses, by the presence and the dynamics of the vascularization inside the mass [80]. By adding a quantitative approach, it may even be possible to differentiate not only thrombi from tumors, but also benign from malignant tumors [79] (Fig. 11, Ref. [79]).

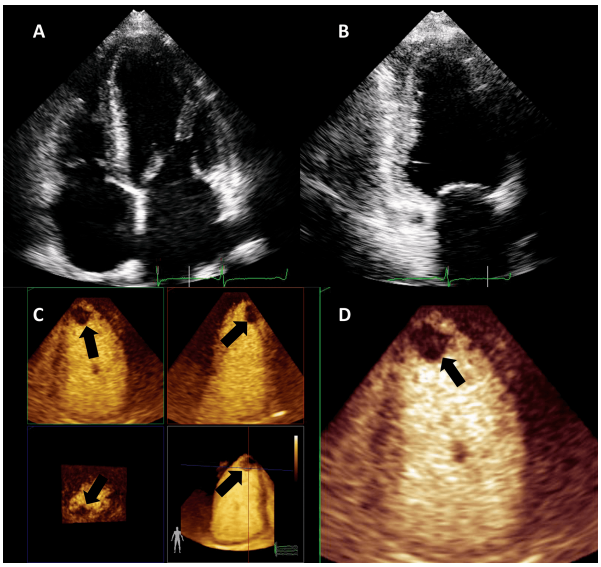


Fig. 10. Detection of an apical thrombus in a patient with severely depressed EF. Native images in apical 4 (A) and 2 chambers (B) do not demonstrate the presence of an apical mass. (C) 3D contrast-enhanced echocardiography demonstrating an apical filling defect (thrombus-arrows). (D) Apical 2-chambers 2D contrast-enhanced image, the thrombus is present in the apical LV. Images modified with permission from Strachinaru *et al*. [79].

4.5 Cardiac Contrast-Enhanced Ultrasound in Children and Adolescents

In children, image quality in transthoracic echocardiography is usually considered to be significantly better than in adults, due to body size, depth of the region of interest and degree of soft tissue hydration. This allows for the use of high-resolution high-frequency transducers, and also implies that the use of ultrasound enhancing agents is less frequent. However, echocardiographic images are not always diagnostic in this patient population, and other imaging modalities also tend to be used less than in adults (cardiac computed tomography being a radiation imaging technology, and cardiac magnetic resonance often requiring sedation for smaller children). All the clinical applications described above (LVO, myocardial contrast, intracardiac mass detection) have potential indications in pediatric patients [81,82]. MEE has been described in the detection of coronary involvement in Kawasaki disease or in congenital heart disease [81,83,84]. In complex congenital heart disease, the acoustic window may be limited by post-surgical anatomy and the interposition of strong reflectors (implanted material). In these patients however, there is a clear benefit from the precise delineation and measurement of the right and left ventricle, which may have unusual shapes [82].

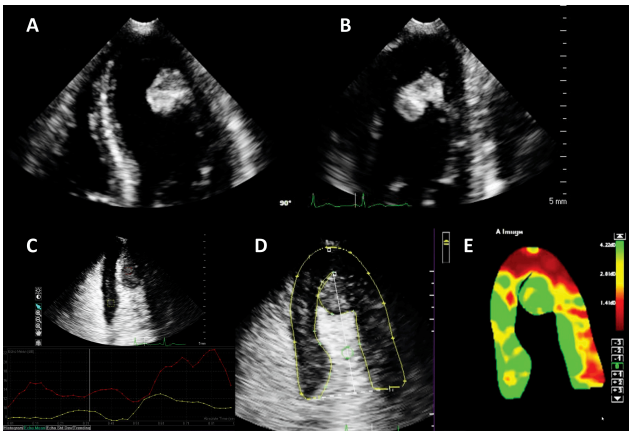


Fig. 11. Hyperechoic and hypermobile mass in a normal LV. The image quality is good, and the mass is hyperechoic. (A) 4-chambers view. (B) 2-chambers view. (C) Signal intensity quantification after a flash-replenishment cycle. The replenishment of the mass is similar to the interventricular septum, but with higher intensity, signalling the presence of a capillary vascularisation, with a higher density than the myocardium. (D,E) Parametric map of signal intensity, demonstrating the same features of the mass. In this patient the mass was finally diagnosed as a hypervascular metastasis from a lung carcinoma. Images modified with permission from Strachinaru *et al*. [79].

The safety of UEA in children has been a subject for debate [13,85–87]. In 2016 the FDA removed the intracardiac shunt contraindication from all UEA labels. There are remaining concerns, in particular in congenital patients with large right-to-left shunting, that the UEA microbubbles may directly enter the arterial circulation, potentially inducing microvascular obstruction [13]. There are no current studies on the safety of UEA in patients under the age of 5.

For these reasons, the use of contrast agents is currently considered safe only in pediatric and congenital heart disease patients older than 5, without large right-to-left shunts. Further studies are needed in this direction, and a thorough benefit/risk assessment should be performed in each case. Optimal dosing of UEA in children corresponds to body weight.

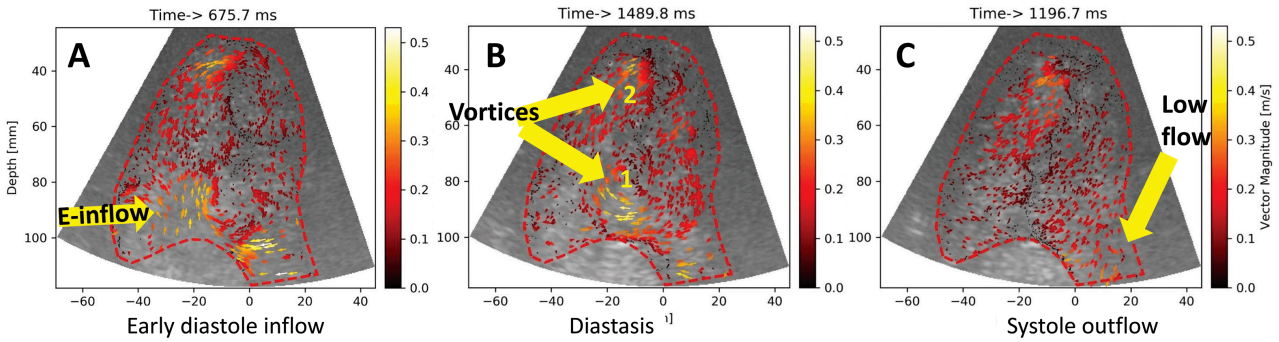
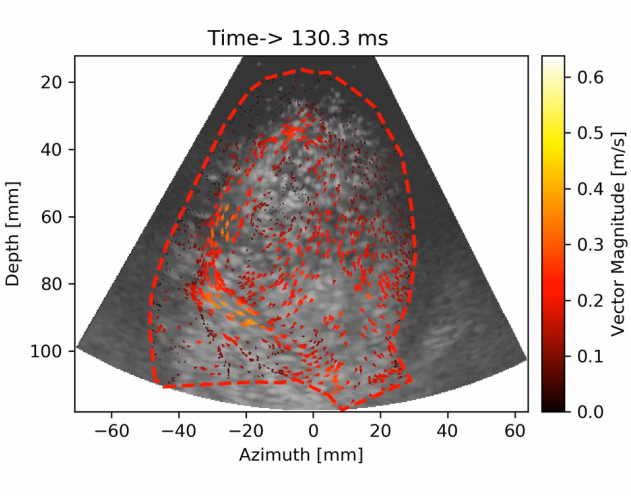


Fig. 12. High frame rate echo particle velocimetry (HFR echoPIV) in a heart failure patient. (A) early diastolic inflow, corresponding to the Doppler E wave; (B) intraventricular flow in mid-diastole, during diastasis; (C) Flow during mid-systole. Inflow, outflow direction and magnitude can be visualized and quantified. Rotational flow (vortices) can also be seen and measured. Source: personal collection.

4.6 Emerging Applications

4.6.1 Intracardiac Flow Tracking and Quantification

Blood flow in the heart is classically imaged using colour Doppler, which has some inherent disadvantages: angle-dependency, relatively low frame rate, low velocity range and semi-quantitative nature. New ultrasound techniques, generally referred to as vector flow imaging, can estimate the location, direction and magnitude of the velocity vectors that describe flow in a region of interest. One such technique, Echo-Particle Image Velocimetry (echoPIV), tracks the speckle of ultrasound contrast agent (UEA) microbubbles [88,89]. These intricate echoPIV flow fields may offer additional meaningful insights, such as derived quantities (vorticity, circulation, kinetic energy, kinetic energy dissipation) [90]. The precise clinical meaning of these derived parameters still needs to be investigated. One of the limitations of conventional echoPIV is the relatively low frame rates permitted by conventional line-scanning based ultrasound imaging (maximum ~100 Hz). In contrast, high frame rate (HFR) echoPIV, using diverging-wave transmit sequences, allows for frame rates in the kHz range and makes tracking of fast flow in the left ventricle (LV) possible [90–93] (Fig. 12, Video 9). However, these methods are still in development as clinical high frame rate imaging is still far from implementation [94].



Video 9. High frame rate echoPIV in a heart failure patient. Individual bubbles are tracked in their motion, and their direction and velocity represented with arrows. This is both a qualitative and quantitative method, allowing to estimate velocity anywhere in the region of interest (here the LV end-diastolic contour, traced with a red dotted line). The movie corresponds to Fig. 12. The embedded movie may also be viewed at https://doi.org/10.31083/j.rcm2306202.

4.6.2 Ultrasound Targeted Microbubble Destruction (UTMD) and Derived Applications

Recent studies demonstrated that targeted drug and gene delivery can be done non-invasively. Genes or other substances may be incorporated on the surface of custom-generated microbubbles, or even inside the shell, and then destroyedas they reach the target area by using high-energy ultrasound pulses [95,96] (Fig. 13). Because the UEA microbubbles are purely intravascular, delivery occurs to the vascular endothelium, but the ultrasound field and the energy generated by cavitation of the microbubbles can facilitate transfection into the extravascular tissue also [97–99]. Ongoing work is directed towards improving transfection of the drugs/genes into the target cells.

High-MI ultrasound pulses induce cavitation, which can increase local blood flow through the generation of nitric oxide [100]. This particular effect of UTMD has been recently used to improve perfusion in sickle-cell anemia [101], and in the myocardium in animal models [102].

4.6.3 Sonothrombolysis

High-MI ultrasound pulses have the potential to disrupt and dissolve intravascular thrombi [103–106]. Prelimi-

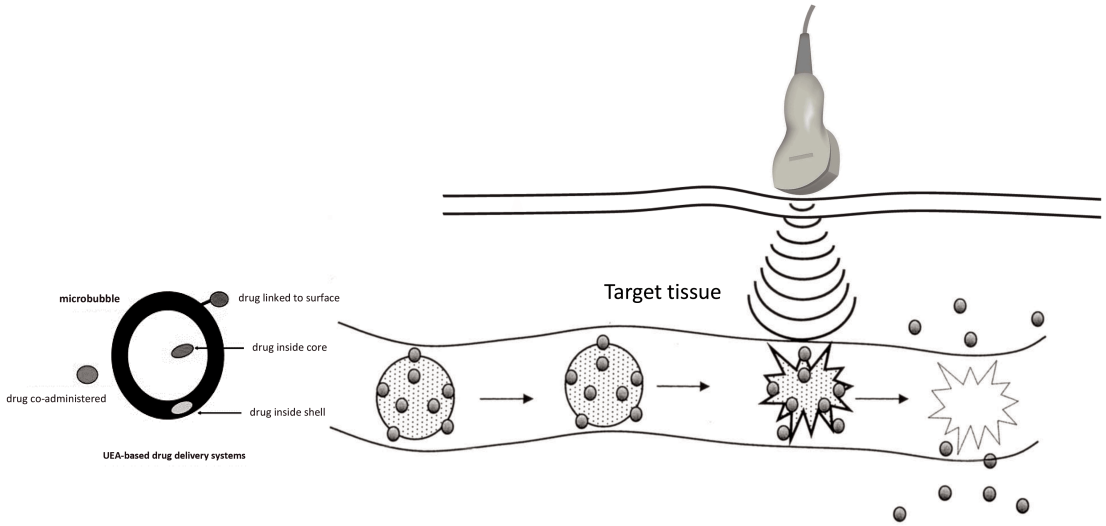


Fig. 13. Theoretical principle of ultrasound targeted microbubble destruction (UTMD) applications. The bubbles can be "fitted" with a drug/gene/other marker, which can be released at the target site by destroying the bubbles with high-MI pulses. Source: personal collection.

nary studies have demonstrated the recanalization of thrombosed vessels, without the adjunction of any drug treatment [103]. Preliminary clinical studies in ST-elevation myocardial infarction (STEMI) patients showed that guided high-MI pulses improve early recanalization rates and restore microvascular flow, reducing the infarct size [106]. Future and ongoing studies focus on the use of sonothrombolysis in acute coronary syndromes and stroke.

4.6.4 Molecular Imaging

UEA are distributed strictly intravascular. By attaching certain ligands on their surface, they can target the surface of dysfunctional endothelium in certain diseased areas. This is achieved through modified UEA microbubbles, which are relatively easy to engineer and in large quantities. By using contrast-specific imaging modalities, and imaging after the normal time of clearance of the free contrast microbubbles, the diseased area can be selectively highlighted [107]. This method has been used in myocardial ischemia, allograft rejection, myocarditis and angiogenesis [108–111].

All these cutting-edge applications of MEE in the diagnosis and treatment of heart disease are mostly at the stage of early clinical translation, but they have shown clinical promise and are gaining momentum through the innovative work of several research groups.

4.7 Safety

Following their initial clinical use in the 90's, ultrasound enhancing agents underwent a period of limited use, induced by fears of adverse events [112]. However, subsequent large studies demonstrated that in various settings (inpatients, outpatients, critically ill, rest or stress testing), no excess in mortality or myocardial infarction was observed when compared to control populations [113,114]. In critically ill patients on mechanical circulatory support devices data has only been reported in a few small single-center reports [20,115,116]; however, none of these studies noted supplementary adverse events in this patient population. Furthermore, contrast-enhanced stress echocardiography was not associated with an increase in adverse events [117–119].

Rare (between 1/1000 and 1/10000 patients) side effects have been noted with contrast agents, usually mild and transient (headache, nausea, dizziness, paraesthesia, taste disturbances or reactions at the injection site). Serious allergic reactions have a very low incidence [120] (considered low-risk, with an incidence of 0.005–0.015%).

Therefore, the clinical use of MEE is considered very safe in all its applications. Meanwhile the FDA has lifted the contraindications initially issued in the 2007 "black box warning" [112]. The only absolute contraindications persisting today are in patients with known or suspected large intracardiac shunting and those with hypersensitivity to the UEA.

Intracoronary administration is also considered contraindicated, despite its systematic and uneventful use in hypertrophic cardiomyopathy patients undergoing septal ablation.

It is recommended that all personnel in contact with a patient during any MEE study should be familiar with early identification of an allergic reaction and the appropriate treatment. Allergy kits including auto-injectable epinephrine should be available and easily accessible [7, 13].

5. Conclusions

Contrast-enhanced echocardiography is a mature technique, with an established safety profile allowing for its routine clinical use. Yet, in spite of extensive clinical experience and research, contrast-enhanced echocardiography remains underused, largely due to insufficient experience of the clinicians and unjustified fear of adverse effects. Through this review we covered the current and future perspectives of MEE, which we hope will facilitate the understanding and incorporation of this method in everyday clinical practice.

Author Contributions

MS gathered the data and drafted the manuscript, FJtC provided part of the illustrations and baseline data, contributed to the writing of the manuscript and reviewed it for key content.

Ethics Approval and Consent to Participate

All patients included in this review as clinical illustrations gave their informed consent for the anonymous use of their clinical data and echocardiographic images.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Medical Advisory Secretariat. Use of contrast agents with echocardiography in patients with suboptimal echocardiography: an evidence-based analysis. Journal: Ontario Health Technology Assessment Series. 2010; 10: 1–17.

[2] Oh JK, Pellikka PA, Panza JA, Biernat J, Attisano T, Manahan BG, *et al*. Core Lab Analysis of Baseline Echocardiographic Studies in the STICH Trial and Recommendation for Use of Echocardiography in Future Clinical Trials. Journal of the American Society of Echocardiography. 2012; 25: 327–336.

[3] Strachinaru M, Papadopoulou B, Damry N, Laureys M, Samyn S. Contrast echocardiography detecting a rare abnormal venous connection. European Heart Journal: Cardiovascular Imaging. 2015; 16: 813–813.

[4] 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. Journal of the American College of Cardiology. 1984; 3: 14–20.

[5] Feinstein SB, Cheirif J, Ten Cate FJ, Silverman PR, Heidenreich PA, Dick C, *et al*. Safety and efficacy of a new transpulmonary ultrasound contrast agent: Initial multicenter clinical results. Journal of the American College of Cardiology. 1990; 16: 316–324.

[6] 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. Journal of the American College of Cardiology. 2009; 53: 802–810.

[7] 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. European Heart Journal: Cardiovascular Imaging. 2017; 18: 1205–1205af.

[8] Vos HJ, Dollet B, Versluis M, de Jong N. Nonspherical Shape Oscillations of Coated Microbubbles in Contact with a Wall. Ultrasound in Medicine & Biology. 2011; 37: 935–948.

[9] Kaufmann BA, Wei K, Lindner JR. Contrast Echocardiography. Current Problems in Cardiology. 2007; 32: 51–96.

[10] Malm S, Frigstad S, Sagberg E, Larsson H, Skjaerpe T. Accurate and reproducible measurement of left ventricular volume and ejection fraction by contrast echocardiography: a comparison with magnetic resonance imaging. Journal of the American College of Cardiology. 2004; 44: 1030–1035.

[11] Hoffmann R, von Bardeleben S, ten Cate F. Assessment of Systolic Left Ventricular Function: a Multi-centre Comparison of Cineventriculography, Cardiac Magnetic Resonance Imaging, Unenhanced and Contrast-enhanced Echocardiography. ACC Current Journal Review. 2005; 14: 33–34.

[12] 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. Journal of the American College of Cardiology. 2006; 47: 121–128.

[13] 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. Journal of the American Society of Echocardiography. 2018; 31: 241–274.

[14] Rakhit DJ, Becher H, Monaghan M, Nihoyannopoulos P, Senior R. The clinical applications of myocardial contrast echocardiography. European Journal of Echocardiography. 2007; 8: S24–S29.

[15] Chahal NS, Senior R. Clinical Applications of Left Ventricular Opacification. JACC: Cardiovascular Imaging. 2010; 3: 188–196.

[16] Becher H, Tiemann K, Schlosser T, Pohl C, Nanda Nc, Averkiou MA, *et al*. Improvement in Endocardial Border Delineation Using Tissue Harmonic Imaging. Echocardiography. 1998; 15: 511–517.

[17] Senior R, Soman P, Khattar RS, Lahiri A. Improved endocardial visualization with second harmonic imaging compared with fundamental two-dimensional echocardiographic imaging. American Heart Journal. 1999; 138: 163–168.

[18] 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. The American Journal of Cardiology. 2006; 98: 1110–1114.

[19] Nucifora G, Marsan NA, Holman ER, Siebelink HJ, van Werkhoven JM, Scholte AJ, *et al*. Real-time 3-dimensional echocardiography early after acute myocardial infarction: Incremental value of echo-contrast for assessment of left ventricular function. American Heart Journal. 2009; 157: 882.e1–882.e8.

[20] Schinkel AFL, Akin S, Strachinaru M, Muslem R, Soliman OII, Brugts JJ, *et al*. Safety and feasibility of contrast echocardiography for the evaluation of patients with HeartMate 3 left ventricular assist devices. European Heart Journal: Cardiovascular Imaging. 2018; 19: 690–693.

[21] Caiani E. Improved Semiautomated Quantification of Left Ventricular Volumes and Ejection Fraction Using 3-Dimensional

Echocardiography with a Full Matrix-array Transducer: Comparison with Magnetic Resonance Imaging. Journal of the American Society of Echocardiography. 2005; 18: 779–788.
[22] 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. European Heart Journal: Cardiovascular Imaging. 2015; 16: 233–270.
[23] Corona-Villalobos CP, Sorensen LL, Pozios I, Chu L, Eng J, Abraham MR, *et al*. Left ventricular wall thickness in patients with hypertrophic cardiomyopathy: a comparison between cardiac magnetic resonance imaging and echocardiography. The International Journal of Cardiovascular Imaging. 2016; 32: 945–954.
[24] Urbano-Moral JA, Gonzalez-Gonzalez AM, Maldonado G, Gutierrez-Garcia-Moreno L, Vivancos-Delgado R, De Mora-Martin M, *et al*. Contrast-Enhanced Echocardiographic Measurement of Left Ventricular Wall Thickness in Hypertrophic Cardiomyopathy: Comparison with Standard Echocardiography and Cardiac Magnetic Resonance. Journal of the American Society of Echocardiography. 2020; 33: 1106–1115.
[25] Hoffmann R, von Bardeleben S, Barletta G, Pasques A, Kasprzak J, Greis C, *et al*. Comparison of Two- and Three-Dimensional Unenhanced and Contrast-Enhanced Echocardiographies Versus Cineventriculography Versus Cardiac Magnetic Resonance for Determination of Left Ventricular Function. The American Journal of Cardiology. 2014; 113: 395–401.
[26] Reilly JP, Tunick PA, Timmermans RJ, Stein B, Rosenzweig BP, Kronzon I. Contrast echocardiography clarifies uninterpretable wall motion in intensive care unit patients. Journal of the American College of Cardiology. 2000; 35: 485–490.
[27] 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. European Journal of Echocardiography. 2009; 10: 933–940.
[28] Thomas JD, Rubin DN. Tissue harmonic imaging: why does it work? Journal of the American Society of Echocardiography. 1998; 11: 803–808.
[29] Soman P, Swinburn J, Callister M, Stephens NG, Senior R. Apical hypertrophic cardiomyopathy: Bedside diagnosis by intravenous contrast echocardiography. Journal of the American Society of Echocardiography. 2001; 14: 311–313.
[30] Strachinaru M, Huurman R, Schinkel AFL. Functional imaging in echocardiography can sometimes replace direct structure visualization. European Heart Journal: Cardiovascular Imaging. 2019; 20: 241–241.
[31] Ogbogu PU, Rosing DR, Horne MK. Cardiovascular Manifestations of Hypereosinophilic Syndromes. Immunology and Allergy Clinics of North America. 2007; 27: 457–475.
[32] Chow C, Lim K, Wu L, Leong-Poi H. Isolated Left Ventricular Noncompaction Enhanced by Echocontrast Agent. Circulation. 2007; 116: e90–e91.
[33] Yalonetsky S, Agmon Y, Lessick J. Contrast Echocardiographic Imaging of Left Ventricular Diverticulum in Adult Patients. Journal of the American Society of Echocardiography. 2007; 20: 198.e1–198.e3.
[34] Lee DZJ, Chan RH, Montazeri M, Hoss S, Adler A, Nguyen ET, *et al*. Left Ventricular Apical Aneurysms in Hypertrophic Cardiomyopathy: Equivalent Detection by Magnetic Resonance Imaging and Contrast Echocardiography. Journal of the American Society of Echocardiography. 2021; 34: 1262–1272.
[35] Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, *et al*. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. European Heart Journal. 2021; 42: 373–498.
[36] Doukky R, Donenberg MJ, Parker J, Kaplan J, Travers C, Soble JS, *et al*. Use of ultrasound enhancing agents in transesophageal echocardiography to improve interpretive confidence of left atrial appendage thrombus. Echocardiography. 2019; 36: 362–369.
[37] von der Recke G, Schmidt H, Illien S, Lüderitz B, Omran H. Use of transesophageal contrast echocardiography for excluding left atrial appendage thrombi in patients with atrial fibrillation before cardioversion. Journal of the American Society of Echocardiography. 2002; 15: 1256–1261.
[38] Bernier M, Abdelmoneim SS, Stuart Moir W, Eifert Rain SSJ, Chandrasekaran K, Ammash NM, *et al*. CUTE-CV: a Prospective Study of Enhanced Left Atrial Appendage Visualization with Microbubble Contrast Agent Use during Transesophageal Echocardiography Guided Cardioversion. Echocardiography. 2013; 344: 1091–1097.
[39] 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. Cardiovascular Ultrasound. 2013; 11: 1.
[40] Kaul S, Senior R, Dittrich H, Raval U, Khattar R, Lahiri A. Detection of Coronary Artery Disease with Myocardial Contrast Echocardiography: comparison with 99mTc-sestamibi single-photon emission computed tomography. Circulation. 1997; 96: 785–792.
[41] Senior R, Janardhanan R, Jeetley P, Burden L. Myocardial Contrast Echocardiography for Distinguishing Ischemic from Nonischemic first-Onset Acute Heart Failure: insights into the mechanism of acute heart failure. Circulation. 2005; 112: 1587–1593.
[42] 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: Cardiovascular Imaging. 2008; 1: 145–152.
[43] Rainbird AJ, Mulvagh SL, Oh JK, McCully RB, Klarich KW, Shub C, *et al*. Contrast dobutamine stress echocardiography: Clinical practice assessment in 300 consecutive patients. Journal of the American Society of Echocardiography. 2001; 14: 378–385.
[44] Jung PH, Rieber J, Störk S, Hoyer C, Erhardt I, Nowotny A, *et al*. Effect of contrast application on interpretability and diagnostic value of dobutamine stress echocardiography in patients with intermediate coronary lesions: comparison with myocardial fractional flow reserve. European Heart Journal. 2008; 29: 2536–2343.
[45] 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. Journal of the American College of Cardiology. 2009; 53: 32–38.
[46] Larsson MK, Da Silva C, Gunyeli E, Ilami AAB, Szummer K, Winter R, *et al*. The potential clinical value of contrast-enhanced echocardiography beyond current recommendations. Cardiovascular Ultrasound. 2016; 14: 2.
[47] Lerakis S, Kalogeropoulos AP, El-Chami MF, Georgiopoulou VV, Abraham A, Lynch SA, *et al*. Transthoracic Dobutamine

Stress Echocardiography in Patients Undergoing Bariatric Surgery. Obesity Surgery. 2007; 17: 1475–1481.
[48] Wake R, Takeuchi M, Yoshitani H, Miyazaki C, Otani S, Yoshiyama M, *et al*. Role of Contrast-Enhanced Dobutamine Stress Echocardiography in Predicting Outcome in Patients with Known or Suspected Coronary Artery Disease. Echocardiography. 2006; 23: 642–649.
[49] 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. Circulation: Cardiovascular Imaging. 2013; 6: 202–209.
[50] Lindner JR, Song J, Jayaweera AR, Sklenar J, Kaul S. Microvascular rheology of Definity microbubbles after intra-arterial and intravenous administration. Journal of the American Society of Echocardiography. 2002; 15: 396–403.
[51] Kaul S, Jayaweera AR. Coronary and myocardial blood volumes: noninvasive tools to assess the coronary microcirculation? Circulation. 1997; 96: 719–724.
[52] 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? Journal of the American College of Cardiology. 1998; 32: 252–260.
[53] Janardhanan R, Moon JCC, Pennell DJ, Senior R. Myocardial contrast echocardiography accurately reflects transmurality of myocardial necrosis and predicts contractile reserve after acute myocardial infarction. American Heart Journal. 2005; 149: 355–362.
[54] 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: 473–483.
[55] Elhendy A, O'Leary EL, Xie F, McGrain AC, Anderson JR, Porter TR. Comparative accuracy of real-time myocardial contrast perfusion imaging and wall motion analysis during dobutamine stress echocardiography for the diagnosis of coronary artery disease. Journal of the American College of Cardiology. 2004; 44: 2185–2191.
[56] Jayaweera AR, Wei K, Coggins M, Bin JP, Goodman C, Kaul S. Role of capillaries in determining CBF reserve: new insights using myocardial contrast echocardiography. American Journal of Physiology-Heart and Circulatory Physiology. 1999; 277: H2363–H2372.
[57] 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. Journal of the American College of Cardiology. 2006; 48: 2168–2177.
[58] 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. European Journal of Echocardiography. 2009; 10: 26–35.
[59] 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. Journal of the American College of Cardiology. 2013; 62: 1353–1361.
[60] Shimoni S, Zoghbi WA, Xie F, Kricsfeld D, Iskander S, Gobar L, *et al*. Real-time assessment of myocardial perfusion and wall motion during bicycle and treadmill exercise echocardiography: comparison with single photon emission computed tomography. Journal of the American College of Cardiology. 2001; 37: 741–747.
[61] Chiou KR, Huang WC, Lin SL, Hsieh PL, Liu CP, Tsay DG, *et al*. Real-time dobutamine stress myocardial contrast echocardiography for detecting coronary artery disease: correlating abnormal wall motion and disturbed perfusion. The Canadian Journal of Cardiology. 2004; 20: 1237–1243.
[62] Gaibazzi N, Reverberi C, Squeri A, De Iaco G, Ardissino D, Gherli T. Contrast Stress Echocardiography for the Diagnosis of Coronary Artery Disease in Patients with Chest Pain but without Acute Coronary Syndrome: Incremental Value of Myocardial Perfusion. Journal of the American Society of Echocardiography. 2009; 22: 404–410.
[63] Gaibazzi N, Rigo F, Reverberi C. Detection of Coronary Artery Disease by Combined Assessment of Wall Motion, Myocardial Perfusion and Coronary Flow Reserve: a Multiparametric Contrast Stress-Echocardiography Study. Journal of the American Society of Echocardiography. 2010; 23: 1242–1250.
[64] Shah BN, Chahal NS, Bhattacharyya S, Li W, Roussin I, Khattar RS, *et al*. The Feasibility and Clinical Utility of Myocardial Contrast Echocardiography in Clinical Practice: Results from the Incorporation of Myocardial Perfusion Assessment into Clinical Testing with Stress Echocardiography Study. Journal of the American Society of Echocardiography. 2014; 27: 520–530.
[65] 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. Journal of the American College of Cardiology. 2013; 61: 2446–2455.
[66] Xiu J, Cui K, Wang Y, Zheng H, Chen G, Feng Q, *et al*. Prognostic Value of Myocardial Perfusion Analysis in Patients with Coronary Artery Disease: a Meta-Analysis. Journal of the American Society of Echocardiography. 2017; 30: 270–281.
[67] Gaibazzi N, Porter T, Lorenzoni V, Pontone G, De Santis D, De Rosa A, *et al*. Effect of Coronary Revascularization on the Prognostic Value of Stress Myocardial Contrast Wall Motion and Perfusion Imaging. Journal of the American Heart Association. 2017; 6: e006202.
[68] 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: 950–956.
[69] 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: 538–544.
[70] Dwivedi G, Janardhanan R, Hayat SA, Swinburn JM, Senior R. Prognostic Value of Myocardial Viability Detected by Myocardial Contrast Echocardiography Early after Acute Myocardial Infarction. Journal of the American College of Cardiology. 2007; 50: 327–334.
[71] Funaro S, La Torre G, Madonna M, Galiuto L, Scarà A, Labbadia A, *et al*. Incidence, determinants, and prognostic value of reverse left ventricular remodelling after primary percutaneous coronary intervention: results of the Acute Myocardial Infarction Contrast Imaging (AMICI) multicenter study. European Heart Journal. 2009; 30: 566–575.
[72] Shah BN, Khattar RS, Senior R. The hibernating myocardium: current concepts, diagnostic dilemmas, and clinical challenges in the post-STICH era. European Heart Journal. 2013; 34: 1323–1336.
[73] 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. Circula-

tion. 2001; 103: 2560–2565.
[74] Vogel R, Indermühle A, Reinhardt J, Meier P, Siegrist PT, Namdar M, *et al*. The quantification of absolute myocardial perfusion in humans by contrast echocardiography: algorithm and validation. Journal of the American College of Cardiology. 2005; 45: 754–762.
[75] 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. Journal of the American College of Cardiology. 2004; 43: 257–264.
[76] Thanigaraj S, Schechtman KB, Pérez JE. Improved Echocardiographic Delineation of Left Ventricular Thrombus with the Use of Intravenous second-Generation Contrast Image Enhancement. Journal of the American Society of Echocardiography. 1999; 12: 1022–1026.
[77] Mansencal N, Nasr IA, Pillière R, Farcot J, Joseph T, Lacombe P, *et al*. Usefulness of Contrast Echocardiography for Assessment of Left Ventricular Thrombus after Acute Myocardial Infarction. The American Journal of Cardiology. 2007; 99: 1667–1670.
[78] Weinsaft JW, Kim RJ, Ross M, Krauser D, Manoushagian S, LaBounty TM, *et al*. Contrast-Enhanced Anatomic Imaging as Compared to Contrast-Enhanced Tissue Characterization for Detection of Left Ventricular Thrombus. JACC: Cardiovascular Imaging. 2009; 2: 969–979.
[79] Strachinaru M, Damry N, Duttmann R, Wauthy P, Catez E, Lutea M, *et al*. Ultrasound Contrast Quantification for the Diagnosis of Intracardiac Masses. JACC: Cardiovascular Imaging. 2016; 9: 747–750.
[80] Kirkpatrick JN, Wong T, Bednarz JE. Differential diagnosis of cardiac masses using contrast echocardiographic perfusion imaging. ACC Current Journal Review. 2004; 13: 25.
[81] Zilberman MV, Witt SA, Kimball TR. Is there a role for intravenous transpulmonary contrast imaging in pediatric stress echocardiography? Journal of the American Society of Echocardiography. 2003; 16: 9–14.
[82] Kutty S, Biko DM, Goldberg AB, Quartermain MD, Feinstein SB. Contrast-enhanced ultrasound in pediatric echocardiography. Pediatric Radiology. 2021; 51: 2408–2417.
[83] Kinoshita Y, Suzuki A, Nakajima T, Ono Y, Arakaki Y, Kamiya T. Collateral vessels assessed by myocardial contrast echocardiography in patients with coronary artery lesions after Kawasaki disease. Heart and Vessels. 1996; 11: 203–210.
[84] Kutty S, Olson J, Danford CJ, Sandene EK, Xie F, Fletcher SE, *et al*. Ultrasound contrast and real-time perfusion in conjunction with supine bicycle stress echocardiography for comprehensive evaluation of surgically corrected congenital heart disease. European Heart Journal: Cardiovascular Imaging. 2012; 13: 500–509.
[85] McMahon CJ, Ayres NA, Bezold LI, Lewin MB, Alonzo M, Altman CA, *et al*. Safety and Efficacy of Intravenous Contrast Imaging in Pediatric Echocardiography. Pediatric Cardiology. 2005; 26: 413–417.
[86] Ntoulia A, Anupindi SA, Back SJ, Didier RA, Hwang M, Johnson AM, *et al*. Contrast-enhanced ultrasound: a comprehensive review of safety in children. Pediatric Radiology. 2021; 51: 2161–2180.
[87] 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. The American Journal of Cardiology. 2013; 112: 1039–1045.
[88] Hong G, Pedrizzetti G, Tonti G, Li P, Wei Z, Kim JK, *et al*. Characterization and Quantification of Vortex Flow in the Human Left Ventricle by Contrast Echocardiography Using Vector Particle Image Velocimetry. JACC: Cardiovascular Imaging. 2008; 1: 705–717.
[89] Abe H, Caracciolo G, Kheradvar A, Pedrizzetti G, Khandheria BK, Narula J, *et al*. Contrast echocardiography for assessing left ventricular vortex strength in heart failure: a prospective cohort study. European Heart Journal: Cardiovascular Imaging. 2013; 14: 1049–1060.
[90] Mele D, Smarrazzo V, Pedrizzetti G, Capasso F, Pepe M, Severino S, *et al*. Intracardiac Flow Analysis: Techniques and Potential Clinical Applications. Journal of the American Society of Echocardiography. 2019; 32: 319–332.
[91] Toulemonde MEG, Corbett R, Papadopoulou V, Chahal N, Li Y, Leow CH, *et al*. High Frame-Rate Contrast Echocardiography: in-Human Demonstration. JACC: Cardiovascular Imaging. 2018; 11: 923–924.
[92] Voorneveld J, Keijzer LBH, Strachinaru M, Bowen DJ, Goei JSL, Ten Cate F, *et al*. High-Frame-Rate Echo-Particle Image Velocimetry can Measure the High-Velocity Diastolic Flow Patterns. Circulation: Cardiovascular Imaging. 2019; 12: e008856.
[93] Voorneveld J, Keijzer LBH, Strachinaru M, Bowen DJ, Mutluer FO, van der Steen AFW, *et al*. Optimization of Microbubble Concentration and Acoustic Pressure for Left Ventricular High-Frame-Rate EchoPIV in Patients. IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control. 2021; 68: 2432–2443.
[94] Strachinaru M, Voorneveld J, Keijzer LBH, Bowen DJ, Mutluer FO, Cate FT, *et al*. Left ventricular high frame rate echo-particle image velocimetry: clinical application and comparison with conventional imaging. Cardiovascular Ultrasound. 2022. (in press)
[95] Christiansen JP, French BA, Klibanov AL, Kaul S, Lindner JR. Targeted tissue transfection with ultrasound destruction of plasmid-bearing cationic microbubbles. Ultrasound in Medicine & Biology. 2003; 29: 1759–1767.
[96] Juffermans LJM, Dijkmans PA, Musters RJP, Visser CA, Kamp O. Transient permeabilization of cell membranes by ultrasound-exposed microbubbles is related to formation of hydrogen peroxide. American Journal of Physiology-Heart and Circulatory Physiology. 2006; 291: H1595–H1601.
[97] Xie A, Wu MD, Cigarroa G, Belcik JT, Ammi A, Moccetti F, *et al*. Influence of DNA-Microbubble Coupling on Contrast Ultrasound–Mediated Gene Transfection in Muscle and Liver. Journal of the American Society of Echocardiography. 2016; 29: 812–818.
[98] 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: 1022–1026.
[99] Chen S, Shimoda M, Chen J, Grayburn PA. Stimulation of adult resident cardiac progenitor cells by durable myocardial expression of thymosin beta 4 with ultrasound-targeted microbubble delivery. Gene Therapy. 2013; 20: 225–233.
[100] 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. Circulation: Cardiovascular Imaging. 2015; 8: e002979
[101] Belcik JT, Davidson BP, Xie A, Wu MD, Yadava M, Qi Y, *et al*. Augmentation of Muscle Blood Flow by Ultrasound Cavitation is Mediated by ATP and Purinergic Signaling. Circulation. 2017; 135: 1240–1252.
[102] Moccetti F, Belcik T, Latifi Y, Xie A, Ozawa K, Brown E, *et al*. Flow Augmentation in the Myocardium by Ultrasound Cavitation of Microbubbles: Role of Shear-Mediated Purinergic Signaling. Journal of the American Society of Echocardiography. 2020; 33: 1023–1031.e2.
[103] Chen X, Leeman JE, Wang J, Pacella JJ, Villanueva FS. New

Insights into Mechanisms of Sonothrombolysis Using Ultra-High-Speed Imaging. Ultrasound in Medicine &Amp; Biology. 2014; 40: 258–262.

[104] Xie F, Slikkerveer J, Gao S, Lof J, Kamp O, Unger E, *et al*. Coronary and Microvascular Thrombolysis with Guided Diagnostic Ultrasound and Microbubbles in Acute ST Segment Elevation Myocardial Infarction. Journal of the American Society of Echocardiography. 2011; 24: 1400–1408.

[105] Xie F, Gao S, Wu J, Lof J, Radio S, Vignon F, *et al*. Diagnostic ultrasound induced inertial cavitation to non-invasively restore coronary and microvascular flow in acute myocardial infarction. PLoS ONE. 2013; 8: e69780.

[106] Mathias W, Tsutsui JM, Tavares BG, Xie F, Aguiar MOD, Garcia DR, *et al*. Diagnostic Ultrasound Impulses Improve Microvascular Flow in Patients with STEMI Receiving Intravenous Microbubbles. Journal of the American College of Cardiology. 2016; 67: 2506–2515.

[107] Lindner JR. Molecular imaging of cardiovascular disease with contrast-enhanced ultrasonography. Nature Reviews Cardiology. 2009; 6: 475–481.

[108] Mott B, Packwood W, Xie A, Belcik JT, Taylor RP, Zhao Y, *et al*. Echocardiographic Ischemic Memory Imaging through Complement-Mediated Vascular Adhesion of Phosphatidylserine-Containing Microbubbles. JACC: Cardiovascular Imaging. 2016; 9: 937–946.

[109] Wu W, Zhang Z, Zhuo L, Zhou L, Liu P, He Y, *et al*. Ultrasound Molecular Imaging of Acute Cellular Cardiac Allograft Rejection in Rat with T-Cell–Specific Nanobubbles. Transplantation. 2013; 96: 543–549.

[110] Steinl DC, Xu L, Khanicheh E, Ellertsdottir E, Ochoa-Espinosa A, Mitterhuber M, *et al*. Noninvasive Contrast-Enhanced Ultrasound Molecular Imaging Detects Myocardial Inflammatory Response in Autoimmune Myocarditis. Circulation: Cardiovascular Imaging. 2016; 9: e004720.

[111] Wang J, Qin B, Chen X, Wagner WR, Villanueva FS. Ultrasound Molecular Imaging of Angiogenesis Using Vascular Endothelial Growth Factor-Conjugated Microbubbles. Molecular Pharmaceutics. 2017; 14: 781–790.

[112] Main ML, Goldman JH, Grayburn PA. Thinking outside the "Box"—the Ultrasound Contrast Controversy. Journal of the American College of Cardiology. 2007; 50: 2434–2437.

[113] 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. Journal of the American College of Cardiology. 2008; 51: 1704–1706.

[114] Main ML, Ryan AC, Davis TE, Albano MP, Kusnetzky LL, Hibberd M. Acute Mortality in Hospitalized Patients Undergoing Echocardiography with and without an Ultrasound Contrast Agent (Multicenter Registry Results in 4,300,966 Consecutive Patients). The American Journal of Cardiology. 2008; 102: 1742–1746.

[115] Bennett CE, Tweet MS, Michelena HI, Schears GJ, Mulvagh SL. Safety and Feasibility of Contrast Echocardiography for ECMO Evaluation. JACC: Cardiovascular Imaging. 2017; 10: 603–604.

[116] Fine NM, Abdelmoneim SS, Dichak A, Kushwaha SS, Park SJ, Mulvagh SL. Safety and Feasibility of Contrast Echocardiography for LVAD Evaluation. JACC: Cardiovascular Imaging. 2014; 7: 429–430.

[117] Timperley J, Mitchell ARJ, Thibault H, Mirza IH, Becher H. Safety of contrast dobutamine stress echocardiography: a single center experience. Journal of the American Society of Echocardiography. 2005; 18: 163–167.

[118] Tsutsui JM, Elhendy A, Xie F, O'Leary EL, McGrain AC, Porter TR. Safety of dobutamine stress real-time myocardial contrast echocardiography. Journal of the American College of Cardiology. 2005; 45: 1235–1242.

[119] 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. The Journal of the American Society of Echocardiography. 2008; 21: 1202–1206.

[120] International Collaborative Study of Severe Anaphylaxis. Risk of anaphylaxis in a hospital population in relation to the use of various drugs: an international study. Pharmacoepidemiology and Drug Safety. 2003; 12: 195–202.