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Assessing the Kinetics of Microbubble Appearance in Cirrhotic Patients Using Transthoracic Saline Contrast-Enhanced Echocardiography

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Abstract

Background—The number of cardiac cycles that need to be reviewed by echocardiography before a significant intrapulmonary shunt can be excluded remains unclear.

Methods—We retrospectively identified patients with cirrhosis who underwent technetium-99m-labeled macroaggregated albumin scanning. The kinetics of bubble appearance after the injection of agitated saline during transthoracic echocardiograms were assessed in these patients.

Results—For the 64 eligible patients, the mean $\pm$ SD age was 56 ± 9 years. The median (IQR) shunt fraction by radionuclide scanning was 7.7 % (2.8 – 19.9%). Microbubbles were seen in the left atrium (LA) and left ventricle (LV) at a median (IQR) of 4 (2–5) and 4 (2–5) beats, respectively. The number of heart cycles before microbubbles appeared in the LA or LV was inversely associated with the nuclear scanning shunt fraction ($R = -0.42$, $p = 0.001$, $R = -0.46$, $p < 0.001$, respectively). If no microbubbles were detected by heart cycle 7, the shunt fraction was uniformly less than 3%. Patients with arterial oxygen < 60 mmHg, compared to ≥ 60 mmHg, had earlier appearance of microbubbles in the left heart chambers (2.6 ± 1.9 versus 4.0 ± 2.3 beats, $p = 0.046$).

Conclusions—In patients with advanced cirrhosis suspected of having hepatopulmonary syndrome, a greater shunt fraction during nuclear scanning was associated with more pronounced hypoxemia and a prompt and more intense appearance of microbubbles in the left-sided heart chambers. Patients with a shunt fraction above 3% have microbubbles in the LA or LV at some point during the first seven heart cycles.

Keywords

Hepatopulmonary syndrome; contrast-enhanced echocardiography; intrapulmonary shunt; radionuclide scanning

Introduction

The hepatopulmonary syndrome (HPS) is a disorder characterized by the presence of intrapulmonary vascular dilations (IPVDs) that lead to hypoxemia in patients with liver disease, portal hypertension, or congenital portosystemic shunts¹. The prevalence of HPS in cirrhotic patients listed for liver transplant varies from 4–32%² and its presence affects the quality of life and doubles the risk of death³; therefore, recognition of the HPS is essential, particularly in patients in whom liver transplantation is a therapeutic option.

The dilation of pulmonary capillaries – called IPVDs – from the usual diameter of 8–10 microns⁴ to 15–60 microns^{5,6} leads to right-to-left shunting, ventilation-perfusion inequality, and O₂ diffusion limitation with resultant hypoxemia⁷. For purposes of defining the HPS,

abnormal arterial oxygenation is designated as a resting alveolar-arterial oxygen gradient (A-a O₂ gradient) on room air of 15 mmHg if 64 years or 20 mmHg if older, in the absence of other vascular or pulmonary parenchymal abnormalities^{1,8}.

Defining features of the HPS include an increased A-a O₂ gradient and evidence of IPVDs⁹ for which agitated saline contrast-enhanced echocardiography is a sensitive screening test^{1,9}. Microbubbles (>10 microns in diameter) are generated by agitating normal saline. This agitated solution is then injected in a peripheral vein and a stream of microbubbles opacify the right heart chambers, under normal circumstances to be later filtered by or dissolved in the pulmonary capillary bed^{10,11}. However, in the presence of IPVDs or another source of right-to-left shunt, the microbubbles can traverse or bypass the pulmonary vasculature, appearing in the left heart chambers.

A more pronounced right-to-left shunt has been associated with greater mortality in cirrhotic patients¹² but there are limited data on whether saline contrast-enhanced echocardiography can help quantify this shunt. In addition, a fundamental unanswered question regards the number of cardiac cycles that need to be reviewed by echocardiography before a significant intrapulmonary shunt can be excluded or the total number of cycles over which bubble appearance should be quantitated. This study examines this issue by characterizing the kinetics of bubble appearance in the left-sided cardiac chambers and comparing these patterns to the results of radionuclide scanning (technetium-99m-labeled macroaggregated albumin [Tc-99m MAA]) in a series of patients with advanced liver disease.

Methods

Patient Selection

This retrospective study was approved by the Cleveland Clinic Institutional Review Board (study number 15–1354). Written informed consent was waived. Based on the intent to study the kinetics of microbubble appearance in the HPS, a total of 101 patients were selected by querying the Cleveland Clinic billing database for the interval October 2005 – September 2015. Patients were eligible if they carried International Classification of Diseases (ICD)-10 code K74 (fibrosis and cirrhosis of the liver) with its respective sub-codes and/or K7681 (hepatopulmonary syndrome), and the Current Procedural Terminology (CPT) code 78428 (diagnostic nuclear medicine procedures on the cardiovascular system).

Patients were included if they have liver disease and the presence of both a transthoracic echocardiogram (TTE) with agitated saline contrast and a Tc-99m MAA radionuclide scanning for review. TTE with agitated saline contrast was ordered to screen for HPS and radionuclide scanning to quantify the shunt. Exclusion criteria included: a) the nuclear test was not found (n=5), b) TTE with bubbles was not available for offline review (n=14), c) the echocardiographic window did not allow an adequate visualization of microbubbles (n=3), d) the first image of recorded video showed microbubbles in the RA (n=10), and e) fewer than 8 heart cycles were recorded after the appearance of microbubbles in the RA (n=5). We reviewed medical records in all patients to confirm the diagnosis and identify the etiology of cirrhosis. In our series, 42 patients had cirrhosis confirmed by liver biopsy, while the rest

had the diagnosis made by liver specialists based on clinical findings and results of imaging studies.

Demographic features, type of liver disease, TTE, and radionuclide scan results were recorded as were results of arterial blood gas (ABG) analysis by radial artery puncture in the sitting position and on room air (n=46). The alveolar-arterial O₂ gradient was determined.

Echocardiography

Following current guidelines, trained sonographers and experienced cardiologists respectively performed and interpreted the echocardiograms¹³. All echocardiograms were reviewed offline by trained investigators (A.R.T., W.D., M.M.P.) who were unaware of the results of the radionuclear scanning. We used commercially available software (Syngo Dynamics Workplace, Siemens Healthcare GmbH, Erlangen, Germany) for the echocardiographic determinations. Measured parameters include RA and left atrial (LA) areas at end-systole, RV and left ventricular (LV) areas at end-diastole, LV ejection fraction and right ventricular systolic pressure (RVSP)^{13,14}. RV size and function were determined visually and by measuring the RV basal diameter and tricuspid annular plane systolic excursion, respectively.

Digital captures of the agitated saline injection were carefully assessed regarding the kinetics of bubble appearance. Saline contrast-enhanced TTE was routinely performed in the left lateral decubitus position after placing an intravenous line and rapidly injecting 8 mL of freshly agitated saline mixed with 1 mL of blood and 0.5 mL of air, while simultaneously recording 2D-echocardiography in the four-chamber apical view^{15,16}. Heart cycles were consecutively numbered from 1 to 12, considering number 1 the first R-to-R electrocardiographic interval during which microbubbles were initially noted in the RA. Whenever possible 12 cardiac cycles were captured from the time microbubbles were first observed entering the RA; nevertheless, in 5 patients the recording stopped at some point between cardiac cycles 8 to 12, potentially related to the fact that in all of them, microbubbles in the left heart chambers were noted between heart cycles 1 to 4.

For each cardiac cycle, the presence and density of microbubbles in the LA and/or LV (on a scale from 0 to 5) was assessed by a study investigator based on several published grading systems^{17–21}, where 0 = no microbubbles in the left cardiac chambers, 1 = <5 microbubbles (minor), 2 = 5 but <30 microbubbles (mild), 3 = 30 but <60 microbubbles (moderate), 4 = 60 microbubbles without outlining the endocardium (significant); and 5 = 60 microbubbles, with clear definition of the endocardium (full LA or LV opacification). For each patient, the heart cycle (after microbubbles were seen in the RA) in which microbubbles were first noted both in the LA and LV was recorded, as was the number of beats required for microbubbles to completely opacify the LA and LV. If microbubbles in the left heart chambers were noted in any of the first 3 cardiac cycles, we reviewed the transesophageal and/or intracardiac echocardiograms when available.

Radionuclide Scanning

Radionuclide scanning was ordered at the discretion of the treating physicians and was performed at a median (IQR) of 0 (0–1.8) months after the echocardiogram with agitated

saline. Radionuclide scanning macroaggregated albumin scanning was obtained after the administration of Tc-99m MAA (20 microns, 2–4 mCi) into a peripheral upper extremity vein while the patient was sitting. In the presence of IPVDs, macroaggregated albumin is not fully retained in the pulmonary capillary bed, travelling to the brain and other systemic organs. Radioactive counts were measured using the geometric mean of the Tc-99m counts in the cerebrum and lungs, in order to calculate the extrapulmonary shunt fraction, a method that assumes that the brain receives 13% of the total systemic blood flow²². In addition, the presence of radioactive uptake in the kidneys, thyroid, spleen, liver, and salivary glands was documented.

Statistical Analysis

Continuous variables were summarized using mean $\pm$ standard deviation or median and interquartile range (IQR) when appropriate. Numerical variables were compared using t-test and categorical variables with Chi-square test. The degree of association between two variables was tested using the Pearson correlation coefficient. Receiver operating characteristic curves with Youden's index was used as a criterion for selecting the optimal cut-off point of a continuous variable of interest. All p values were reported as two-tailed. A p value of <0.05 was considered statistically significant. Statistical analyses were performed using the statistical package SPSS version 17 (IBM; Armonk, N.Y., USA) and MedCalc version 14.12.2 (MedCalc Software bvba, Ostend, Belgium).

Results

Patient Demographic Features and ABG Determinations

For the 64 eligible patients, the mean $\pm$ SD age was 56 ± 9 years and 34 (53%) were men (Table 1). Arterial blood gases on room air were available in 46 (72%) patients and showed a mean PaO_2 of 62.6 ± 11.3 mmHg (range 41–92 mmHg) and an A-a O_2 gradient of 46.8 ± 16.2 mmHg (range 6.5–80.2 mmHg). All but two patients had an elevated aged-adjusted A-a O_2 gradient⁹ with the degree of hypoxemia classified as mild ($\text{PaO}_2 \geq 80$ mmHg, $n=5$, 11%), moderate (PaO_2 60–79 mmHg, $n=19$, 43%), severe (PaO_2 50–59 mmHg, $n=15$, 34%), and very severe ($\text{PaO}_2 < 50$ mmHg, $n=5$, 11%)⁹.

Shunt Assessment by Radionuclide Scanning

The median (IQR) shunt fraction by radionuclide scanning was 7.7 % (2.8 – 19.9%). A total of 46 (72%), 41 (64%), 41 (64%), 39 (61%) and 34 (53%) patients had a shunt fraction ≥ 3 , ≥ 4 , ≥ 5 , ≥ 6 , ≥ 7 %, respectively. In the entire cohort, radioactive uptake was clearly noticeable in the brain, kidneys, thyroid and liver in 50 (78%), 19 (30%), 5 (8%) and 5 (8%) patients, respectively.

Shunt Detection by Echocardiogram

Selected echocardiographic determinations are presented in Table 1. None of the patients had 2-D or Doppler evidence of anatomic intracardiac shunt (including PFO, or atrial or ventricular septal defects). Microbubbles were seen in the LA and LV at a median (IQR) of 4 (2–5) and 4 (2–5) beats, respectively. In the third and fifth cardiac cycles, microbubbles were noted in either the LA or LV in 27 (42%) and 47 (73%) patients, respectively (Table 2,

Figure 1). The LA was completely opacified with microbubbles at some point during the recording in 44 (69%) patients, at a median (IQR) of 7 (6–9) heart cycles. The LV entirely filled with microbubbles in 47 (73%) patients at a median (IQR) of 8 (6–10) beats. The density of microbubbles in the LA and LV at each cardiac cycle is shown in Table 2.

The records of the 28 (44%) patients in whom microbubbles were noted in any of the first 3 cardiac cycles were carefully reviewed. Of these 28, 4 subjects had a PFO (2 diagnosed by transesophageal and 2 by intracardiac echocardiograms) while 12 individuals had no evidence of this condition (8 by transesophageal and 4 by intracardiac echocardiograms). These 16 studies (10 transesophageal and 6 intracardiac echocardiograms) were done a median (IQR) of 0 (–2.5 to 1.5) months from the TTE and in all but one (clear interatrial shunt noted by Doppler), agitated saline with Valsalva maneuver was used to assess the presence of an intracardiac shunt. The remaining 12 patients had no further investigations to categorically confirm or exclude a PFO; however, in 4 of these subjects, the results of a subsequent TTE with agitated saline made the presence of PFO very unlikely.

The early (< 3 heart cycles) appearance of microbubbles in the left heart was associated with a larger nuclear scanning shunt fraction when contrasted with the delayed (> 3 cardiac cycles) appearance of microbubbles ($17.3 \pm 10.9\%$ versus $9 \pm 9.5\%$, $p=0.003$). This significant difference persisted when comparing patients with early appearance of microbubbles without evidence of PFO ($n=12$) versus those with delayed appearance of microbubbles in the left heart chambers ($18.5 \pm 10.2\%$ versus $9.1 \pm 9.6\%$, $p=0.007$). Of patients with RVSP ≥ 40 mmHg ($n=21$), 9 (43%) had early microbubbles, a similar percentage to patients with RVSP < 40 mmHg (46%).

The RV/LV area ratio was inversely associated with the number of heart beats to visualize bubbles in the LA ($R=-0.31$, $p=0.02$) and directly associated with the density of bubbles in the LA ($R=0.27$, $p=0.04$). Similarly, the RV basal diameter was inversely associated with number of heart beats to first appearance of bubbles in the LA ($R=-0.28$, $p=0.03$). No significant associations were noted with the RA area, RA/LA area ratio, and parameters derived from the agitated saline study. The left and right atrium and ventricular areas, their ratio, and RVSP were not significantly associated with the nuclear scan shunt fraction.

Relationship between Shunt Detection by Echocardiogram and Radionuclide Scanning

The number of heart cycles in which microbubbles first appeared in the LA or LV was inversely associated with the nuclear scanning shunt fraction ($R=-0.42$, $p=0.001$ and $R=-0.46$, $p<0.001$, respectively). In addition, the number of cardiac cycles until the LA and LV were fully opacified with microbubbles (grade 5) was inversely related to the nuclear shunt fraction ($R=-0.50$, $p<0.001$ and $R=-0.52$, $p<0.001$, respectively).

Table 3 shows the mean $\pm$ SD nuclear scanning shunt fraction based on the presence of microbubbles in the LA and LV during the first 12 cardiac cycles, indicating that the nuclear shunt fraction was greater when microbubbles appeared earlier in the left cardiac chambers. A nuclear scanning shunt fraction > 7.1% was the optimal cut-off to identify the early appearance of microbubbles in the left heart chambers, with a sensitivity of 82% and specificity of 72%. The density of microbubbles in the LA and LV was significantly

associated with the shunt fraction by nuclear scanning, particularly with cardiac cycles five and six (Table 2). Interestingly, the shunt fraction during nuclear scanning was always below 3% when microbubbles were not seen after cardiac cycle 7 (Table 3).

Given the limited number of patients with HPS in the groups with $\text{PaO}_2 \geq 80$ and < 50 mmHg, we collapsed the degree of hypoxemia into two groups ($\text{PaO}_2 \geq 60$ and < 60 mmHg, with 24 and 20 patients, respectively). Patients with more pronounced hypoxemia ($\text{PaO}_2 < 60$ mmHg) had higher nuclear scanning shunt fraction (20.2 ± 11.4 versus 9.2 ± 8.8 %, $p=0.001$) with earlier appearance of microbubbles in the left heart chambers (2.6 ± 1.9 versus 4.0 ± 2.3 beats, $p=0.046$) and earlier complete opacification (grade 5) of the LA (6.6 ± 1.7 versus 8.3 ± 2.0 beats, $p=0.019$). The density of bubbles in the LA at cardiac cycle numbers 3, 5, and 7 was greater in the group of patients with more severe hypoxemia (0.7 ± 0.9 versus 0.3 ± 0.6 , $p=0.04$; 2.8 ± 1.9 versus 1.4 ± 1.6 , $p=0.01$; and 3.9 ± 1.4 versus 2.1 ± 2.0 , $p=0.002$, respectively).

Discussion

The present detailed analysis of the kinetics of microbubble appearance in left-sided cardiac chambers in patients with HPS due to cirrhosis showed that patients with a marked shunt by nuclear scanning have a prompt and pronounced appearance of microbubbles in the LA and LV. In fact, in some patients we saw microbubbles in the left heart chambers during the first 3 heart cycles, after the appearance in the RA, even in the absence of PFO. The density of microbubbles in the LA and LV correlated best with shunt fraction by nuclear scanning on heart cycles 6 to 9. Importantly, if no microbubbles were noted by heart cycle 7, the shunt fraction by nuclear scan was uniformly less than 3% in this series.

Microbubbles were observed in the left cardiac chambers within the first three cardiac cycles in 44% of patients in this series. With the inherent limitations of an extrapolation, a prevalence of 44% exceeds that of PFO in the general population (25–30%)²³, potentially suggesting that early appearance of microbubbles in the left-sided cardiac chambers may occur in patients with pronounced intrapulmonary shunts (as noted in our cohort) and high-output state. In support of this, in our series, a transesophageal or intracardiac echocardiogram ruled out a PFO in 43% of subjects with microbubbles in the left cardiac chambers in any of the first three beats. In this context, our results may challenge the widely accepted notion that the appearance of microbubbles within 3 cardiac cycles of RA appearance specifically indicates an intra-cardiac shunt. These findings underscore the importance of imaging beyond TTE (e.g., transesophageal echocardiography or intracardiac echo) in selected patients, in an attempt to distinguish between intra-cardiac and intrapulmonary shunt²⁴, since this differentiation can have important clinical implications, i.e., a pronounced intrapulmonary shunt may be misclassified a PFO.

In addition to supporting this provocative interpretation of early microbubble appearance, our study also extends inquiry into the echocardiographic features of the HPS by assessing the kinetics of microbubble appearance and by comparing bubble patterns with the results of nuclear scanning. A key finding was a direct association between the density of microbubbles in the LA and LV and the degree of shunting by nuclear scanning, with the

association strongest in cardiac cycles 6 to 8. It is worth mentioning that maximum microbubble density in the LA and LV was noted after cardiac cycle 7 in a quarter of the patients. In an earlier study that assessed bubble intensity in cirrhotic patients, Ferreira et al.²⁵ also found a direct association between the degree of right-to-left shunting assessed by contrast echocardiography and nuclear scanning, though specific patterns of bubble appearance by cardiac cycle were not described.

Lung perfusion scanning with Tc-99m MAA is considered abnormal when the extrapulmonary shunt fraction is 6–7 %^{22,26,27}. This test has a) lower sensitivity (20–96%) than agitated saline-enhanced echocardiography for detecting intrapulmonary vascular dilations^{22,28}, particularly in less severe cases, b) does not distinguish intracardiac from intrapulmonary shunts, and c) exposes subjects to small doses of radiation. Nevertheless, nuclear scanning is particularly useful in quantifying the shunt and determining the contribution to hypoxemia in patients with lung parenchymal abnormalities²⁹. In fact, the extrapulmonary uptake with nuclear scanning is directly proportional to the degree of hypoxemia^{30–32}. More importantly, the extrapulmonary shunt fraction may impact pre- and post-transplant survival as the mean uptake was found to be higher in non-survivors than in survivors^{30,32}.

We observed that a larger RV was associated with an early appearance of bubbles in the LA, which suggests higher preload and hence RV dilation. The sooner microbubbles appeared in left-sided cardiac chambers, the more pronounced the shunt. Of special note is that if no microbubbles were detected by beat seven, patients uniformly had a shunt fraction by nuclear scanning < 3%. This finding supports a practice of evaluating seven cardiac cycles after RV bubble appearance but no more than seven in assessing the presence of an anatomic shunt.

Several limitations of the study warrant comment: First, as echocardiographic assessment study was limited to TTE imaging, it is conceivable that causes of intra-cardiac shunt (e.g., PFO, etc.) might have escaped attention. Still a good proportion of patients had follow-up transesophageal or intracardiac echocardiograms that rule out an intra-cardiac shunt. Second, not all patients had arterial blood gases on room air, thereby limiting the analysis. Finally, selection of eligible patients by ICD-10 codes and the need to have radionuclide scanning may have biased selection and could limit generalizability. In spite of these limitations, our study showed that echocardiography, a test that does not involve radiation and provides valuable cardiovascular information, could also quantify the degree of right-to-left shunt in patients with advanced liver cirrhosis, useful information since a more pronounced right-to-left shunt has been associated with greater hypoxemia and mortality in cirrhotic patients¹². Because an abnormal degree of shunt was never observed in patients with microbubbles beyond seven cardiac cycles, the absence of bubbles beyond this 7-cycle criterion may obviate the need for Tc-99m MAA in such patients.

Conclusions

In summary, in patients with advanced cirrhosis, suspected of having hepatopulmonary syndrome, a more pronounced shunt fraction during nuclear scanning is associated with a

prompt and more intense appearance of microbubbles in the left-sided heart chambers. Patients with a shunt fraction above 3% have microbubbles in the left atrium or ventricle at some point during the first seven heart cycles after the appearance of microbubbles in the right atrium. Confirmatory studies in cirrhotic patients both with and without HPS will further advance understanding of the kinetics of microbubble appearance.

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Abbreviations

ABG	arterial blood gas
ET-1	endothelin-1
HPS	hepatopulmonary syndrome
IQR	interquartile range
IPS	intrapulmonary shunt
LA	left atrium
LV	left ventricle
PaO₂	partial arterial pressure of oxygen
RA	right atrium
RV	right ventricle
ROC	receiver operating characteristic
SaO₂	saturation of oxygen in arterial blood
Tc-99m MAA	technetium-99m-labeled macroaggregated albumin

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Key points

1. Largest study in patients with cirrhosis screened for HPS that investigates the kinetics of microbubbles during agitated saline TTE, using nuclear scanning as comparator.
2. Density and timing of appearance of microbubbles on left heart chamber track with severity of right-to-left shunt on nuclear scanning.
3. Microbubbles can appear in patients with HPS during the first 3 beats, even in the absence of patent foramen ovale, likely related to the high cardiac output and large IPVDs seen in patients with cirrhosis.
4. If microbubbles are seen after heart beat number 7, the shunt by nuclear scanning is minimal with marginal effects on systemic oxygenation.

Figure 1. Percentage of patients with microbubbles in the LA based on the cardiac cycles after the injection of agitated saline

Percentage of patients with no microbubbles in the LA is shown as black columns, while the percentage of those with microbubbles in the LA are represented as gray columns. The superimposed line shows the percentage of patients in whom the LA had maximum density of microbubbles.

Table 1**Patient Characteristics and Echocardiographic Determinations**

Patient Characteristics	Mean $\pm$ SD, n (%)
n	64
Age (years)	56 $\pm$ 9
Male gender	34 (53%)
Body surface area (m ²)	1.95 $\pm$ 0.23
Type of cirrhosis	
- nonalcoholic steatohepatitis	20 (31)
- hepatitis C virus	15 (23)
- alcoholic	12 (19)
- autoimmune hepatitis	4 (6)
- cryptogenic	4 (6)
- primary biliary cirrhosis	2 (3)
- other etiologies	7 (11)
Echocardiographic Determinations	
RA area (cm ²)	15.9 $\pm$ 4.0
LA area (cm ²)	19.8 $\pm$ 4.2
RA/LA area ratio	0.81 $\pm$ 0.15
RV area (cm ²)	22.1 $\pm$ 4.2
RV basal diameter (cm)	3.6 $\pm$ 0.47
LV area (cm ²)	34.5 $\pm$ 6.3
RV/LV area ratio	0.65 $\pm$ 0.11
RV function	
- Normal	61 (95)
- Mildly decreased	3 (5)
LV ejection fraction (%)	61.1 $\pm$ 5.3
RVSP (mmHg)	34 $\pm$ 12
RVSP $\geq$ 40 mmHg.	21 (33)

Abbreviations: LA: left atrium, LV: left ventricle, RA: right atrium, RV: right ventricle, RVSP: right ventricular systolic pressure.

Presence and Density of Microbubbles in the LA and LV at Each Cardiac Cycle after the Visualization of Microbubbles in the RA

Table 2

Cardiac Cycle												
	1	2	3	4	5	6	7	8	9	10	11	12
n	64	64	64	64	64	63	63	60	59	59	59	59
Microbubbles in LA or LV (n (%))	12 (19)	20 (31)	27 (42)	36 (56)	47 (73)	52 (83)	53 (84)	50 (83)	51 (87)	52 (88)	55 (93)	53 (90)
Bubble Density LA (grades 0–5) (number of patients)												
0	55	51	46	35	30	18	17	13	11	10	9	11
1	8	11	8	12	9	6	4	7	7	4	5	4
2	1	2	9	10	13	16	14	8	5	8	6	6
3	0	0	1	4	0	2	2	5	5	6	7	7
4	0	0	0	3	6	5	5	2	3	2	3	3
5	0	0	0	0	6	16	21	25	28	29	29	28
Association with shunt fraction by nuclear test (R, p)	0.15, 0.23	0.13, 0.30	0.22, 0.09	0.48, <0.001	0.67, <0.001	0.70, <0.001	0.71, <0.001	0.73, <0.001	0.71, <0.001	0.69, <0.001	0.69, <0.001	0.68, <0.001
Bubble Density LV (grades 0–5) (number of patients)												
0	53	46	42	29	19	13	10	10	9	7	4	6
1	10	14	14	16	13	13	9	7	6	6	7	6
2	1	4	7	13	13	13	14	9	9	7	8	8
3	0	0	1	2	6	4	3	4	4	8	7	6
4	0	0	0	4	10	8	7	6	6	4	6	6
5	0	0	0	0	3	12	20	24	25	27	27	27
Association with shunt fraction by nuclear test (R, p)	0.13, 0.30	0.31, 0.01	0.25, 0.05	0.55, <0.001	0.59, <0.001	0.71, <0.001	0.73, <0.001	0.68, <0.001	0.68, <0.001	0.67, <0.001	0.66, <0.001	0.68, <0.001

Abbreviations: Y: yes, N: no.

Table 3

Percentage of Shunt by Nuclear Scanning Based on the Presence of Microbubbles in the LA and/or LV at Different Cardiac Cycles Following the Visualization of Microbubbles in the RA

Cardiac cycle												
	1	2	3	4	5	6	7*	8	9	10	11	12
n	64	64	64	64	64	63	63	60	59	59	59	59
Percentage of shunt by nuclear scanning												
Presence of microbubbles Mean $\pm$ SD	16.9 $\pm$ 10.1	17.4 $\pm$ 11.6	16.1 $\pm$ 9.9	16.2 $\pm$ 11.2	15.1 $\pm$ 11.0	14.2 $\pm$ 11.1	13.8 $\pm$ 10.8	14.4 $\pm$ 10.9	13.8 $\pm$ 11.1	13.6 $\pm$ 11.1	13.0 $\pm$ 11.0	12.9 $\pm$ 10.8
No microbubbles (%) Mean $\pm$ SD (range)	11.1 $\pm$ 10.8 (0.9–40.4)	9.8 $\pm$ 9.8 (0.9–32.5)	9.3 $\pm$ 10.8 (0.9–40.4)	6.9 $\pm$ 8.1 (0.9–31.6)	4.03 $\pm$ 4.5 (1.3–16.9)	2.6 $\pm$ 1.6 (0.9–6.9)	2.3 $\pm$ 0.6 (1.3–2.9)	2.1 $\pm$ 0.7 (0.9–2.9)	2.2 $\pm$ 0.6 (1.3–2.9)	2.2 $\pm$ 0.6 (1.3–2.9)	2.1 $\pm$ 0.7 (1.3–2.9)	1.9 $\pm$ 0.8 (0.9–2.9)

* When no microbubbles were observed at heart cycle 7, the nuclear scanning shunt fraction was < 3% in all patients.