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CLINICAL/ORIGINAL PAPER

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

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Aims To determine if perfusion stress echocardiography (PSE) with Imagify™ (perflubutane polymer microspheres) is comparable to stress perfusion imaging using ^{99m}Tc single photon emission computed tomography (SPECT) for coronary artery disease (CAD) detection. PSE is a novel technique for evaluating myocardial perfusion. RAMP (real-time assessment of myocardial perfusion)-1 and -2 were international, Phase 3 trials that evaluated the ability of PSE with Imagify, to detect CAD.

Methods and results Chronic, stable, chest pain patients ($n = 662$) underwent Imagify PSE and gated SPECT imaging at rest and during dipyridamole stress. Independent blinded cardiologists [three PSE readers per trial, and four SPECT readers (one for RAMP-1, three for RAMP-2)] interpreted images. CAD was defined by quantitative coronary angiography or 90-day outcome with clinical review. Accuracy, sensitivity, and specificity were evaluated using non-inferiority analysis (one-sided alpha = 0.025) compared with SPECT. SPECT results for RAMP-1 and -2 were: accuracy (70%, 67%), sensitivity (78%, 61%), and specificity (64%, 76%). Accuracy of all six PSE readers was non-inferior to SPECT (66–71%, $P \leq 0.004$). Four demonstrated non-inferior sensitivity (68–77%, $P \leq 0.002$), three demonstrated non-inferior specificity (72–88%, $P \leq 0.013$). Three PSE readers (RAMP-2) were superior for sensitivity. Two PSE readers (RAMP-1) were superior for specificity. Area under the multi-reader receiver operating characteristics curve (0.72) was equal for both modalities. Majority of adverse events followed dipyridamole dosing, and were mild, transient, and required no treatment.

Conclusions Imagify PSE was well-tolerated. Its diagnostic performance in chest pain patients is comparable with SPECT perfusion imaging.

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Introduction

Invasive coronary angiography (ANGIO) is the gold-standard technique for coronary artery disease (CAD) diagnosis. Because ANGIO is relatively invasive and expensive, with associated morbidity and mortality,[1,2] clinical practice has evolved to risk-stratify patients so that only those with high pre-test probability of disease undergo ANGIO.[3,4]

Echocardiography is currently the non-invasive tool of choice to assess cardiac anatomy and function.[5] Stress echocardiography (SE) and gated single photon emission computed tomography (SPECT) are well-established imaging techniques for CAD detection[6,7] in patients at low to intermediate pre-test risk.[3] Because SPECT detects myocardial perfusion abnormalities, it is likely to be more sensitive than SE for the detection of CAD.[8]

Ultrasound microbubble contrast agents (e.g. Optison™ and Definity™) improved SE image quality for functional assessment.[9] These agents are indicated for patients with suboptimal echocardiograms for left ventricular (LV) opacification, and to improve delineation of LV endocardial borders during resting imaging.[10,11] Their safety and efficacy as diagnostic tools for detecting perfusion and CAD has not been established during stress testing.[3,10,11]

Perflubutane polymer microspheres for injectable suspension (Imagify™) is a novel, biodegradable, synthetic, microsphere ultrasound imaging agent, engineered to be mechanically stronger than currently available ultrasound contrast agents.[12] This mechanical strength allows Imagify to resist destruction by the ultrasound beam during real-time imaging extending visualization time to assess both myocardial perfusion and wall motion.[12] This potentially accounts for the better diagnostic performance of perfusion stress echocardiography (PSE) as compared with non-contrast imaging observed in Phase 2 trials.[13]

We hypothesized that PSE with Imagify would have similar ability to detect CAD as stress ^{99m}Tc SPECT perfusion imaging in stable chest pain patients being evaluated for inducible ischaemia. Two independent multi-center international intra-subject comparison trials of Imagify PSE and stress ^{99m}Tc SPECT perfusion imaging were conducted to assess the diagnostic performance of these modalities in chest pain patients typically referred for non-invasive stress testing.

Methods

Study design

Real-time assessment of myocardial perfusion in echocardiography (RAMP)-1 and -2 were two Phase 3, international, multi-center, stress echocardiography studies, which used perflubutane polymer microspheres (Imagify™, Acusphere, Inc. Watertown, MA, USA) in patients with chest pain. The trials enrolled patients concurrently from independent study-specific sites. Local ethics committees approved study protocols, and all patients provided written informed consent. RAMP-1 and -2 were designed to evaluate non-inferiority of PSE compared with SPECT for CAD detection. Patients received two injections of Imagify, one during rest and the second during vasodilatory stress. Images were interpreted by independent readers completely blinded to all clinical information. There were three PSE readers per study (six PSE readers in total); one SPECT reader for RAMP-1, and three SPECT readers (to allow assessment of SPECT reader variability) for RAMP-2 with the median reader (i.e. reader with intermediate diagnostic performance for a given statistic: accuracy, sensitivity, and specificity) prospectively defined as the comparator.

Patient population

Eligible patients were 18–80-year-old men and non-pregnant/non-lactating women with stable chest pain suggestive of myocardial ischaemia, indicated for vasodilatory stress perfusion imaging. RAMP-1 patients were scheduled for SPECT imaging. RAMP-2 patients had recently undergone or were scheduled to undergo ANGIO. Additionally, 42% of RAMP-1 patients underwent ANGIO. Exclusion criteria were any clinically unstable conditions within 7 days prior to Imagify dosing; acute myocardial infarction (AMI), cerebrovascular accident, or transient ischaemic attack within 30 days of dosing; severe congestive heart failure; significant left main CAD ($\geq$50% stenosis); oxygen saturation $<$90%; or moderate to severe chronic obstructive pulmonary disease. Patients with a prior coronary artery bypass graft (CABG) were excluded from RAMP-2. The intent-to-treat (ITT) population included all patients with an evaluable CAD diagnosis who received Imagify and who had vasodilatory rest/stress SPECT performed on the same day as PSE (87% of patients), or SPECT conducted within 45 days prior to or 15 days following PSE (13% of patients; mean was 12 days prior to PSE).

Imaging agent and image acquisition

Imagify consists of microspheres made of a synthetic biodegradable polymer and phospholipid with microsphere diameter of $\sim$2 μm,[12] and microsphere concentration range of $1.5–2.7 \times 10^9$/mL. Imagify was reconstituted with 5 mL of sterile water for injection. A resulting suspension of 0.04 mL/kg was manually administered by slow (1–10 min) intravenous injection with average duration of 6 min for each imaging session. The rate of injection was dependent on the degree of myocardial enhancement.

For stress imaging, the vasodilator dipyridamole (0.56 mg/kg) was administered as a 4-min intravenous infusion (starting at $t = 0$). Following a 2-min wait ($t = 6$ min), radiopharmaceutical was administered followed by Imagify administration ($t = 7$ min). For PSE imaging, dipyridamole was used exclusively. For stress SPECT, 94% of patients received dipyridamole, 6% received adenosine.

Ninety-five per cent of SPECT imaging was performed with ^{99m}Tc sestamibi and 5% using other isotopes (201-thallium and ^{99m}Tc sestamibi or tetrofosmin or rest/stress tetrofosmin). SPECT imaging occurred 45–90 min after radiopharmaceutical dosing (mean ^{99m}Tc dose of 596 and 993 MBq, for rest and stress, respectively). Gated SPECT images were acquired using a low-energy, high-resolution collimator, without attenuation correction. Ninety per cent of images were acquired using a 180° orbit; 10% were acquired using 360° orbits. All SPECT images were acquired in 64×64 matrix size. SPECT gating was performed at 8 or 16 frames per cardiac cycle. SPECT image reconstruction was conducted using the Yale-CQ software (New Haven, CT, USA) as previously described by an independent central facility (Yale Radionuclide Core Laboratory, New Haven, CT, USA).[14]

All PSE images were acquired using a Sonos 5500 or 7500 (Philips Ultrasound Andover, MA, USA) S3 transducer, and post-processing curve of 2/0/A. Three apical views were imaged using real-time power modulation (operating frequency 2.5 MHz, mechanical index 0.2–0.3, frame rate 20–30 Hz) with destruction impulse duration of 20 ultrasound frames (mechanical index 1.0). Real-time imaging in Ultraharmonics (operating frequency 1.3–3.6 MHz, mechanical index 0.8–1.0, frame rate 20–30 Hz) was followed by triggered end-systolic images (frame rate $\leq$1 Hz) acquired in automated 15 beat sequences for each view. PSE acquisition was performed in 5–10 min to enable imaging in both modes; specific acquisition times varied by patient.

Image analysis and interpretation

For PSE and SPECT, images were stored and presented to blinded readers by a third party. Off-site independent cardiologists who were blinded to all clinical information about the subjects

evaluated all images. Each reader evaluated rest and stress images from a single patient in the same reading session.

For PSE, a 15-segment LV model (six basal-to-mid LV segments, six mid-to-apical LV segments, and three perspectives of the apex) was used. Each segment was evaluated for the presence of a myocardial defect, which was defined as a perfusion abnormality and/or regional wall motion abnormality (RWMA). In absence of wall motion/thickening abnormalities, images that showed uniform myocardial contrast enhancement were considered normal. Defects were assessed as being either fixed (i.e. reduced myocardial contrast enhancement in segments demonstrating WMA at rest with no change at stress) or reversible (i.e. increase in extent or severity of contrast defect between rest and stress, or stress defect alone with or without accompanying WMA). A patient with $\geq$1 segment defect was considered CAD-positive. The presence of a defect in any of the three segments representing the apical cap resulted in a positive score. The resulting model corresponded to a 13-segment SPECT model (six basal-to-mid LV segments, six mid-to-apical LV segments, and one segment for apex).

For SPECT, CAD status evaluation was defined as a myocardial defect (regional perfusion deficit at rest or stress). Defects were assessed for reversibility (i.e. scintigraphic improvement at rest of regional perfusion deficit at stress) and were considered fixed when defect at rest did not change significantly during stress. Images were evaluated on tomographically reconstructed SPECT images along with circumferential profile quantification. Perfusion defects involved $\geq$2 adjacent short-axis slices, or $\geq$2 adjacent vertical or horizontal long-axis slices or WMA in combination with evidence of defect on corresponding regional static slices.

Segmental results for PSE and SPECT were collapsed to three territories (anterior, lateral, and inferoposterior). A patient was considered CAD-positive if they had a defect $\geq$1 territory.

Evaluations of the acoustic window quality (AWQ) from each patient's non-contrast harmonic image were blinded and occurred independently to the evaluations of CAD from PSE images. The AWQ of each image was scored as being good, average, or poor. Images were 'good' when all myocardial territories in each of the three apical views were clearly visible, 'average' when one or more myocardial coronary territory in any of the three apical views was not visualized well, and 'poor', when blinded readers felt quantitative measurements would either be impossible or yield unreliable results. Majority assessment from 3 PSE readers was used in analysis.

Determination of coronary artery disease status and diagnostic test results

Patient CAD status was determined by ANGIO, if available (42% RAMP-1, 99% RAMP-2), including left ventriculography (LVG) in 66% of ANGIO patients. Patients were considered CAD-positive if there was $\geq$70% diameter stenosis in any major epicardial artery or in two smaller coronary artery segments (of which one or more was of medium size) or $\geq$50% diameter stenosis of the left main,[3,15] or RWMA on LVG (*Figure 1*).

Quantitative coronary angiography was used to analyse ANGIO images with the CMS-GFT MEDIS (Leiden, Netherlands) software using standardized methodology developed within the Angiographic Core Laboratory at the Cardiovascular Research Foundation (New York, NY, USA).

In the absence of ANGIO/LVG data, patients' CAD status was determined based on history of AMI (presence of elevated cardiac enzymes or ECG changes) or by an independent nuclear cardiologist (unblinded CAD reviewer), whose evaluation was based on clinical (including cardiovascular history), electrocardiogram, and unblinded SPECT myocardial image data, without access to PSE and SPECT blinded reader interpretation (*Figure 1*). An evaluation of CAD status was not made if the reviewer was uncertain about the presence of CAD.

Disease severity was assessed by global jeopardy score (GJS) using $\geq$70% stenosis cut-off.[16] Only patients without prior CABG, who had

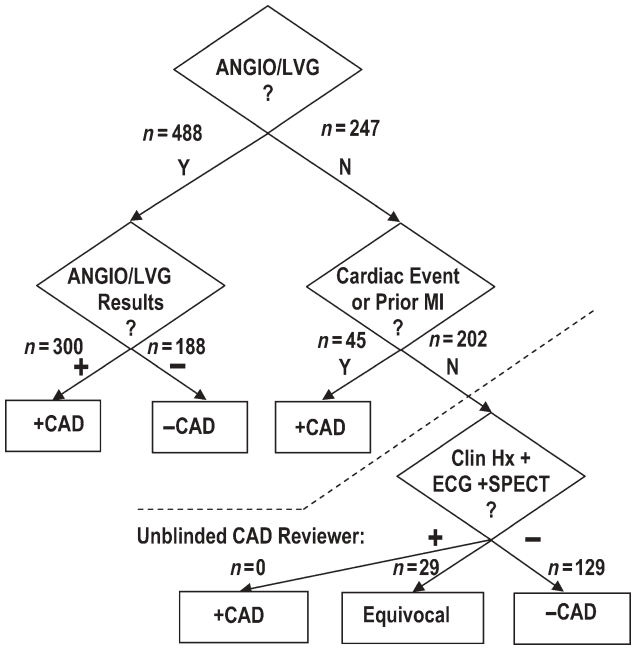

Figure 1 Truth standard schema for patients in RAMP-1 and RAMP-2 trials. ANGIO/LVG was used to define truth, if available. In the absence of ANGIO/LVG, unblinded CAD review and/or clinical history were used. MI, myocardial infarction.

disease determined by ANGIO, were analysed for severity (93 and 368 for RAMP-1 and -2, respectively).

Statistical analysis

Each patient served as his or her own control. Non-inferiority testing of the ratios of PSE to SPECT diagnostic performance for each parameter (i.e. accuracy, sensitivity, and specificity) was evaluated ($>$0.83; one-sided alpha = 0.025) using methods specific for a matched-pair setting.[17] This method considers the ratio of marginal probabilities between two correlated proportions, and allows hypothesis testing of a null value other than 1.0 (as is the case for non-inferiority analysis).

Trends in diagnostic test sensitivity over increasing GJS were tested with the Cochran Armitage test using the following GJS categories: $\geq$0 and $<$10; $\geq$10 and $<$20; $\geq$20 and $<$30; $\geq$30.

χ^2 Tests were used for comparisons of diagnostic performance in single vs. multiple vessel disease. In analyses of modality-specific multi-reader receiver operating characteristics (ROC), the areas under the curves (AUC) were calculated using the trapezoidal rule.

Intra-reader agreement was assessed in duplicate evaluations for 8–26% (depending on study and reader) of the images that were randomly selected. In addition, inter-reader agreement was evaluated for all pairs of readers within and across the two imaging modalities (i.e. within RAMP-1, 6 pairwise comparisons were made between 3 PSE and 1 SPECT reader; in RAMP-2, 15 pairwise comparisons were made between 3 PSE and 3 SPECT readers). Overall mean per cent agreement was calculated for comparisons of PSE to PSE readers, SPECT to SPECT readers, and PSE to SPECT readers for both RAMP-1 and -2 together. Differences between these means were assessed using the Wilcoxon Rank Sum Test.

All analyses were performed using Statistical Analysis Software (SAS®, SAS Institute Inc., Cary, NC, USA) v. 9.1.3.

Results

Population characteristics

From December 2003 to May 2006, RAMP-1 and RAMP-2 concurrently and independently enrolled 321 and 457 patients,

respectively, from 28 sites representing North America, Europe, and Australia. The ITT population (285 and 377 patients in RAMP-1 and -2, respectively) excluded patients with uncertain/missing CAD status ($n = 29$, RAMP-1; $n = 2$, RAMP-2) and missing or ineligible SPECT assessment ($n = 6$, RAMP-1; $n = 78$, RAMP-2). No patients were excluded due to unavailable or unevaluable PSE images. Compared with RAMP-1, RAMP-2 patients had a higher frequency of most cardiac risk factors (*Table 1*) consistent with CAD prevalence of 44 and 58%, respectively.

Table 1 Characteristics of RAMP-1 and RAMP-2 ITT population

	RAMP-1	RAMP-2	*P*-value[a]
n	285	377	
Age, years; mean ± SD	61 ± 10	62 ± 10	0.030
Men, *n* (%)	193 (68)	312 (83)	<0.001
Hypertension, *n* (%)	213 (75)	294 (78)	0.329
Diabetes mellitus, *n* (%)	76 (27)	129 (34)	0.038
Hyperlipidemia, *n* (%)	202 (71)	308 (82)	0.002
Smoking history, *n* (%)			0.009
Never	116 (41)	111 (29)	
Current	48 (17)	82 (22)	
Former	121 (43)	184 (49)	
Previous AMI, *n* (%)	89 (31)	81 (21)	0.005
Previous CABG, *n* (%)	45 (16)	0	NA
Previous PCI, *n* (%)	161 (56)	187 (50)	0.080
BMI (kg/m^2; mean ± SD)	28 ± 5	29 ± 5	0.067
BMI ≥30 kg/m^2, *n* (%)	91 (32)	135 (36)	0.297
CAD[b] (truth standard), *n* (%)	125 (44)	220 (58)	<0.001

[a]*P*-values derived from t-tests and chi-squared tests for continuous and categorical variables, respectively.

[b]CAD status was determined by ANGIO/LVG (RAMP-1, 42%; RAMP-2, 99%), or prior AMI, or was adjucated by independent nuclear cardiologist (details in Methods).

Diagnostic performance of perfusion stress echocardiography vs. single photon emission computed tomography

Accuracy was similar for all six PSE readers (range 66–71%) and the four SPECT readers (range 66–70%) was non-inferior to SPECT (*Table 2*). Sensitivity and specificity, however, varied by reader. PSE reader sensitivity ranged from 50 to 77%, and specificity ranged from 55 to 88%. Similar variations were observed for all SPECT readers. SPECT sensitivity ranged from 57 to 80%, and specificity ranged from 50 to 78%. Sensitivity of four out of six PSE readers was non-inferior to that of the SPECT comparators. Moreover, sensitivity of three PSE readers was also superior to that of the SPECT comparators. Specificity of three out of six PSE readers was non-inferior to that of SPECT with two RAMP-1 PSE readers also demonstrating superior specificity to SPECT.

Due to the heterogeneity of truth standards used in RAMP-1, an analysis was performed to determine if the diagnostic performance of blinded readers was affected by the truth standard. The sensitivity and specificity of blinded readers in RAMP-1 were compared with the subset of RAMP-1 patients whose CAD status was determined by ANGIO/LVG (42%). There were minor insignificant differences in sensitivity and specificity among the PSE readers and in sensitivity for the SPECT reader. In contrast, specificity decreased from 64 to 37% for the SPECT reader in the subset of patients who had only ANGIO as truth standard.

A cut-point of 50% stenosis for CAD is often used in SPECT prognosis studies and has been reported in multiple diagnostic studies.[18] Comparative analysis of two different definitions of angiographic disease (≥50 vs. ≥70% stenosis) revealed that estimates of accuracy varied no more than ±6%, and sensitivity and specificity varied no more than ±8%. Observed differences were similar among PSE and SPECT readers suggesting that both modalities perform similarly using either 50 or 70% stenosis as the CAD criteria.

Table 2 Comparison of the diagnostic test performance of PSE and SPECT readers for CAD detection

Study	Parameter	SPECT comparator[a]	PSE reader 1	PSE reader 2	PSE reader 3
RAMP-1	Accuracy, % (95% CI)	70 (64, 75)	66 (61, 72)	67 (61, 72)	71 (66, 76)
	Rel. ratio (95% CI)		0.95 (0.86, 1.05)	0.96 (0.87, 1.06)	1.02 (0.93, 1.12)
	P-value[b]		0.004	0.002	<0.001
	Sensitivity, % (95% CI)	78 (69, 85)	77 (68, 84)	57 (48, 66)	50 (41, 59)
	Rel. ratio (95% CI)		0.99 (0.88, 1.11)	0.73 (0.63, 0.84)	0.64 (0.54, 0.74)
	P-value		0.002	0.968	>0.999
	Specificity, % (95% CI)	64 (56, 71)	58 (50, 66)	75 (68, 82)	88 (82, 93)
	Rel. ratio (95% CI)		0.91 (0.78, 1.07)	1.18 (1.04, 1.35)	1.38 (1.24, 1.57)
	P-value		0.120	<0.001	<0.001
RAMP-2	Accuracy, % (95% CI)	67 (62, 72)	66 (61, 70)	70 (65, 74)	70 (65, 75)
	Rel. ratio (95% CI)		0.98 (0.89, 1.07)	1.04 (0.96, 1.12)	1.04 (0.96, 1.13)
	P-value		<0.001	<0.001	<0.001
	Sensitivity, % (95% CI)	61 (54, 67)	73 (66, 79)	68 (61, 74)	73 (67, 79)
	Rel. ratio (95% CI)		1.19 (1.08, 1.34)	1.11 (1.01, 1.24)	1.20 (1.09, 1.34)
	P-value		<0.001	<0.001	<0.001
	Specificity, % (95% CI)	76 (68, 82)	55 (47, 63)	72 (64, 79)	66 (58, 73)
	Rel. ratio (95% CI)		0.73 (0.62, 0.85)	0.95 (0.84, 1.06)	0.87 (0.76, 0.98)
	P-value		0.951	0.013	0.259

[a]Diagnostic statistics for the two non-comparator SPECT readers in RAMP-2 were 66 and 67, 57 and 80, and 78 and 50% for accuracy, sensitivity, and specificity, respectively.

[b]$P < 0.025$ indicates non-inferior performance to SPECT comparator.

Diagnostic performance by disease extent and severity

Angiographic severity of disease as indicated by multi-vessel disease was 41% in RAMP-1 and 53% in RAMP-2. All readers had greater sensitivity to detect multi-vessel compared with single-vessel disease (*Figure 2*). Performance varied by reader rather than imaging modality with the highest level of sensitivity among patients with multi-vessel disease for the single RAMP-1 SPECT and PSE reader 1 (95 and 90%, respectively). All readers demonstrated increased sensitivity with increased disease severity (*Figure 3*).

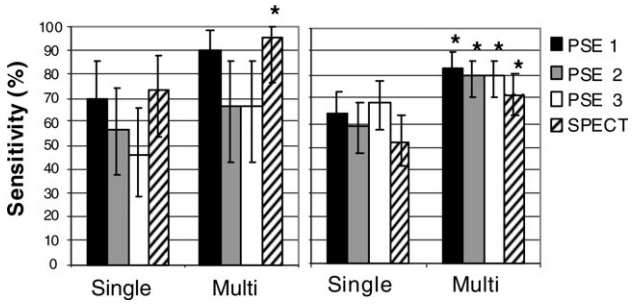

Figure 2 Diagnostic test performance in single and multiple vessel disease. Left and right panels represent RAMP-1 and RAMP-2 data, respectively. *$P < 0.05$, comparison of sensitivity in single vs. multi-vessel disease.

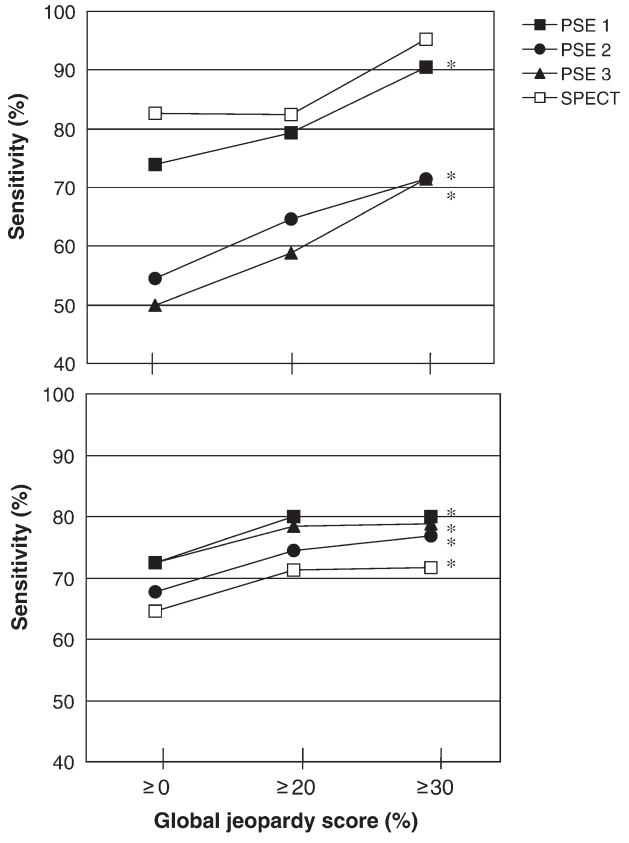

Figure 3 Diagnostic test performance by global jeopardy score. Disease severity was assessed by percentage of left ventricular myocardial mass subtended by stenotic vessels (≥70% stenosis), calculated using a global jeopardy score. Top and bottom panels represent RAMP-1 and RAMP-2 data, respectively. *$P < 0.05$, comparison of sensitivity across global jeopardy score within each reader.

Regional disease detection and localization

Diagnostic performance of each reader for detection of defects in the anterior or the posterior circulation indicated that variations between readers were present. No clear trends between performance and modality or vascular territory were present. The range of sensitivity for anterior and posterior regions was similar for both PSE and SPECT readers (*Figure 4*).

Figure 5 demonstrates representative PSE and SPECT images from the same patient at rest and under dipyridamole stress. Both modalities showed a large apical lateral defect corresponding to left circumflex disease.

Receiver operating characteristic analysis

Multi-reader ROC analysis was used to evaluate the overall performance of PSE and SPECT. All RAMP-1 and -2 readers (six PSE, four SPECT) were included in the analysis (*Figure 6*, top panel). The AUCs were equal (0.72 for both PSE and SPECT) with similar shapes, which indicates that readers from both modalities are making similar trade-offs between sensitivity and specificity. To evaluate the performance of PSE and SPECT to only detect inducible ischaemia, an ROC analysis was performed excluding patients with history of prior AMI (i.e. 26% of patients with fixed wall motion defects). There was a slight decrease in the resulting AUCs (0.69 for PSE and 0.71 for SPECT) (*Figure 6*, bottom panel).

Intra- and inter-reader agreement for perfusion stress echocardiography and single photon emission computed tomography

Intra-reader agreement ranged from 77 to 100% and 81 to 100% for six PSE readers and four SPECT readers, respectively. Inter-reader agreement assessed within both trials ranged from 66 to 80% ($P < 0.001$) for PSE and 73 to 88% ($P < 0.001$) for SPECT readers in RAMP-2. Overall mean per cent agreement for comparisons of PSE to PSE readers was 73% (95% CI; 68, 78) and was 78% (95% CI; 59, 98) for comparisons of SPECT to SPECT readers. Differences between these means were not statistically significant ($P = 0.3642$). Overall mean per cent agreement among readers across the two modalities (i.e. PSE readers compared with SPECT readers) was 68% (95% CI; 66, 70) and was significantly different than mean agreement observed within both modalities ($P < 0.035$).

Acoustic window quality of Imagify perfusion stress echocardiography

AWQ was good for 18% of patients, average for 55% of patients, and poor for 27% of patients. Despite a high proportion of poor AWQ non-contrast images, >99% of all patients undergoing PSE had diagnostic quality contrast images. The estimates of accuracy, sensitivity, and specificity according to AWQ category were within 10% of the estimates determined for the ITT population and indicated no apparent trends in diagnostic performance as related to AWQ.

Safety

No deaths occurred in RAMP-1 or RAMP-2 study. In the combined ITT population ($n = 662$), serious adverse events (SAE) were experienced by four (0.6%) patients. One patient

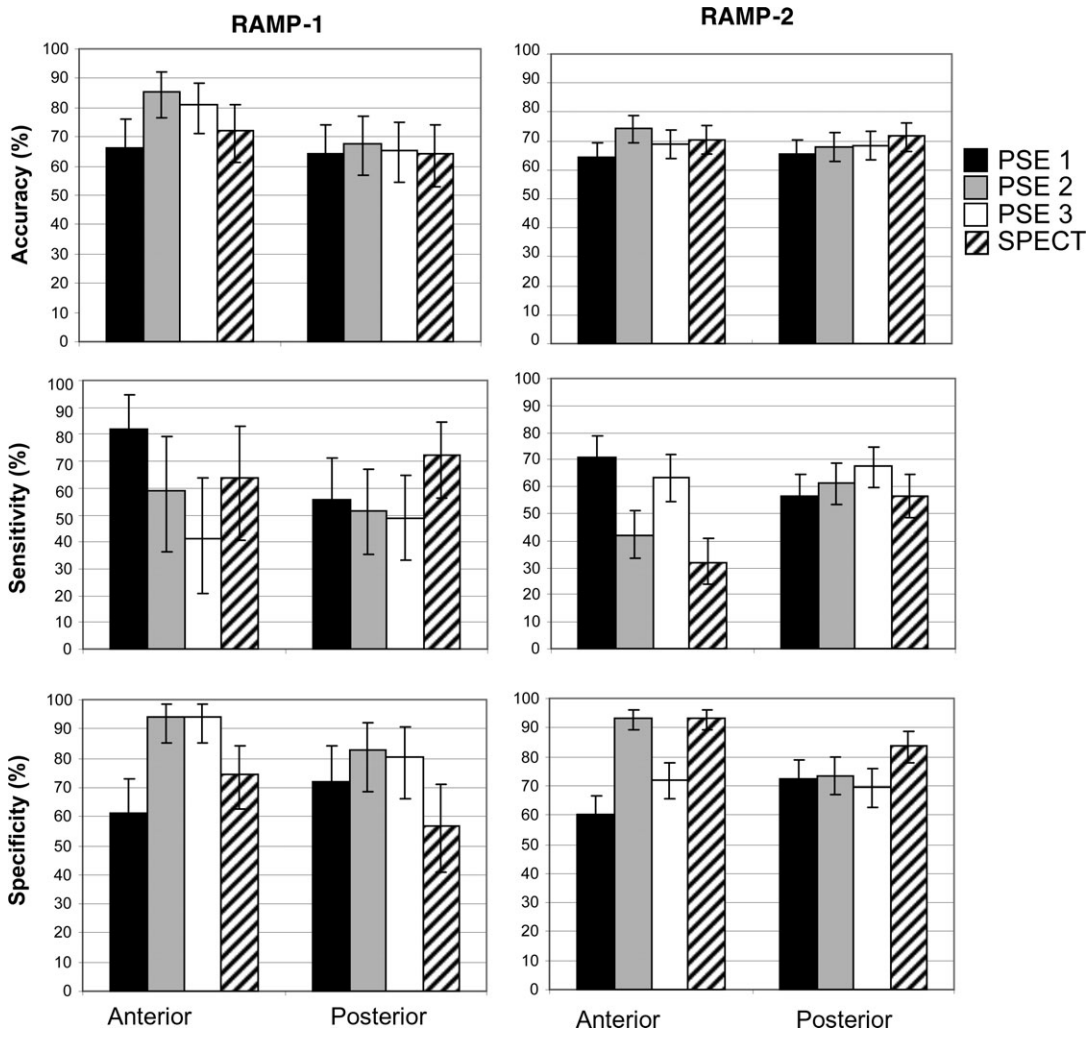

Figure 4 Defect detection and localization by vascular territory. Accuracy, sensitivity, and specificity among PSE and SPECT readers in the detection of coronary artery disease in anterior (LAD) and posterior (RCA and LCx) circulation.

experienced shortness of breath, irritability, and shakiness without any significant decrease in oxygen saturation that was possibly related to hypersensitivity to dipyridamole or Imagify, 1 h after the second dose of Imagify. Shortly thereafter, the patient had episodes of brochospasm and expiratory wheezing. The patient's symptoms were resolved by treatment with aminophylline and hydrocortisone. One patient experienced dizziness, hot flushes, headache, and right-sided chest pain, with 38.6°C fever and chills 3 h after Imagify dosing, which were resolved by treatment with paracetamol. Another two patients experienced SAEs during follow-up (1–5 days after Imagify dosing). One patient had eye pain, visual disturbances, and blurred vision, however, neurological findings on the day after Imagify dosing were normal. One patient had an AMI diagnosed from a rise in cardiac enzymes 2 days after Imagify dosing—the patient was asymptomatic, had no ST elevation, and had undergone diagnostic ANGIO a day earlier. All SAEs were non-life-threatening, occurred at least an hour after Imagify dosing, and resolved without residual effects.

Adverse events (AE) were reported in 454 (69%) patients. The majority of all AEs (>98%) were mild or moderate in severity, were not serious, and resolved without residual effects. Ten per cent of patients reported AEs after the first dose of Imagify, before dipyridamole dosing. The most frequently reported AEs (in $n \geq 5$ patients) prior to dipyridamole were headache (2.6%), flushing (1.8%), and hypotension (0.8%). Overall, the most frequently reported AEs were headache (34%), chest pain (10%), nausea (10%), flushing (9%), and chest discomfort (8%), and mainly occurred following dipyridamole infusion. Investigators were more likely to attribute these AEs to dipyridamole rather than imaging agent.

Mean changes in vital signs (blood pressure, respiratory rate, and body temperature), and oxygen saturation were minimal and did not indicate any untoward trend (data not shown). Laboratory values remained unchanged except for transient mean increases observed in white blood cell (WBC) count and absolute neutrophil count. Mean WBC count increased from $6.7 \times 10^3/\mu L$ at baseline to $9.1 \times 10^3/\mu L$ 2–3 h following stress imaging, which correlated to a transient increase in neutrophils from $4.3 \times 10^3/\mu L$ at baseline to $7.4 \times 10^3/\mu L$ 2–3 h following stress imaging. Both WBCs and neutrophils returned to baseline values at follow-up and were not associated with either febrile responses or acute infections.

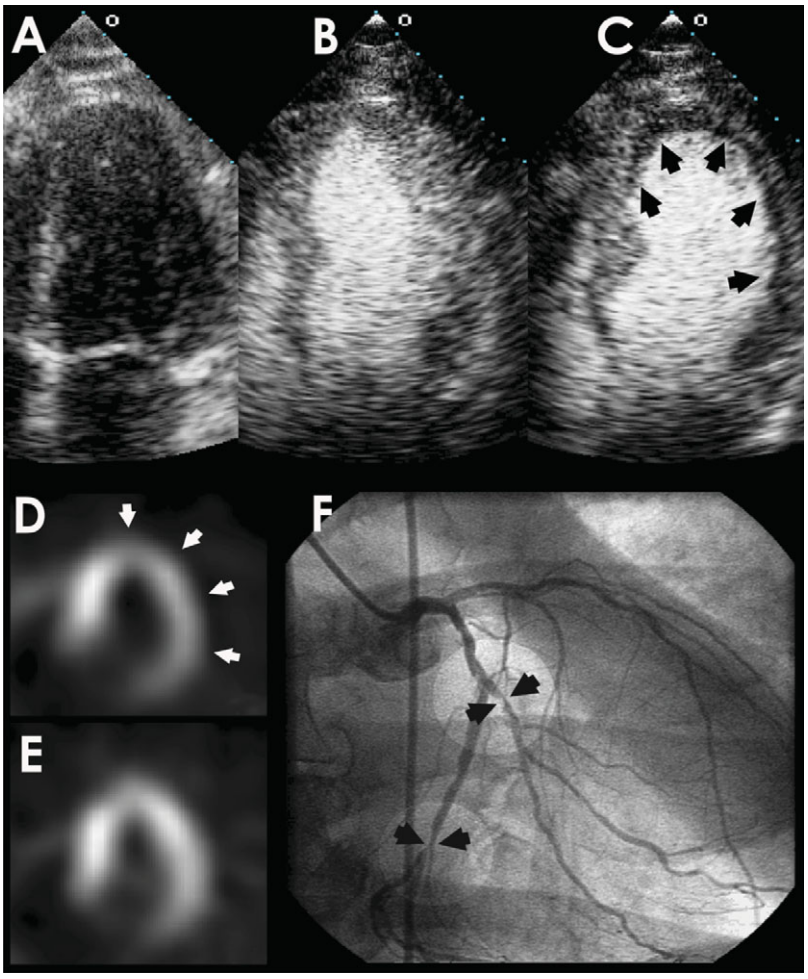

Figure 5 Detection of CAD by PSE. Apical 4-chamber view without contrast and after administration of Imagify at rest (*A* and *B*). Apical lateral (inducible subendocardial) perfusion defect during PSE (*C*), corresponding to similar perfusion defect on horizontal long-axis view in stress SPECT (*D*). SPECT image at rest (*E*). ANGIO with lesions in left circumflex system (*F*). Images were obtained from the same patient at rest and with dipyridamole. Black arrows indicate subendocardium (*C*). Arrows indicate perfusion defects (*C* and *D*) and angiographic lesions (*F*).

Discussion

The results from these two large international trials, which in combination span the entire spectrum of patients typically referred for non-invasive CAD testing, collectively demonstrate that PSE with Imagify has clinically equivalent diagnostic performance to SPECT for the detection of CAD in stable chest pain patients being evaluated for inducible ischaemia. Non-inferiority testing, analysed separately for each trial, and evaluation of ROC curves, analysed collectively, indicated no differences in overall diagnostic performance between the two modalities. Given that the range of values for sensitivity and specificity was similar for both modalities, variations are attributable to a specific reader's performance rather than to imaging modality.

Multi-reader ROC analysis resulted in identical AUCs for PSE and SPECT. The AUC reflects each modality's overall ability to discriminate between diseased and non-diseased patients while allowing for variations in individual blinded reader's disease detection threshold for equivocal cases. Conservative readers have a high threshold for disease detection and thus have lower sensitivity and higher specificity, whereas aggressive readers have a low threshold for diagnosing disease resulting in higher sensitivity and lower specificity. Additional ROC analysis revealed similar AUCs for PSE and SPECT in patients without prior history of AMI. This subgroup of patients likely represents an enriched population of patients without WMAs at rest who had a stress inducible abnormality as the only indication of disease. Because the dipyridamole dose used in RAMP-1 and -2 has been reported to be suboptimal for detection of WMA[19–21] but sufficient for induction of perfusion defects, it is more likely that diagnosis of CAD was often based on the presence of reversible perfusion defects. Taken together, the evidence suggests that PSE is equivalent to SPECT for the detection of myocardial perfusion defects.

More than 99% of all patients undergoing PSE had diagnostic quality images, despite poor AWQ in 27% of patients, indicating robust performance for PSE. The acoustic properties and enhanced stability of Imagify enable PSE imaging to be conducted at higher mechanical indices[13] than imaging using currently approved contrast agents. Higher mechanical index imaging with Imagify allows for better penetration of the ultrasound beam making it easier to scan.

The results of these studies demonstrate comparable performance between PSE using Imagify microspheres and

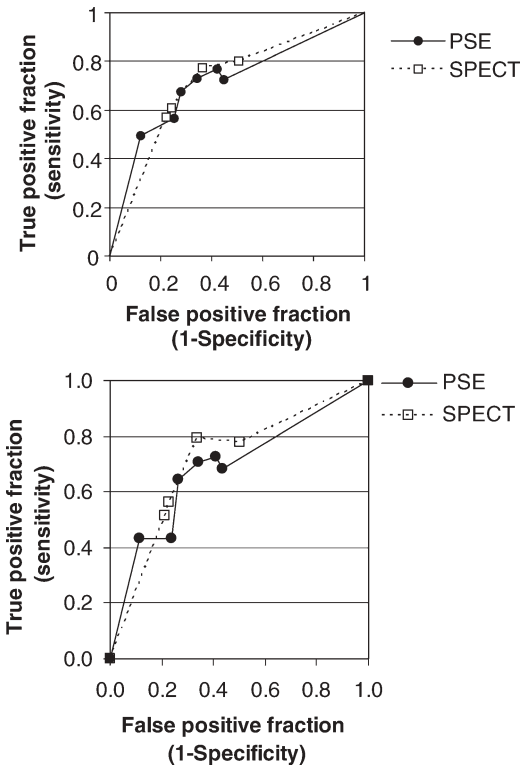

Figure 6 Multi-reader receiver operating characteristics. Values for each Blinded Reader from RAMP-1 and -2 (top) and for the patient subgroup without any prior history of AM (bottom). Modality-specific curves were extrapolated to the theoretical minimum and maximum values. AUCs were 0.72 for both PSE and SPECT (top), and 0.69 for PSE and 0.71 for SPECT (bottom).

gated and quantitative SPECT perfusion imaging and in general, concur with smaller mostly single-centre studies, which have been performed on microbubble agents but using quite different trial and statistical analysis designs.[22–25] The two large, independent multi-centre studies presented here include non-inferiority and multi-reader ROC analyses of imaging data which were obtained from readers who were blinded to all clinical information and who were independent from the sites enrolling the patients. However, these results are consistent with those obtained in similarly independent blinded reads, including results from registration trials.[26,27] Additionally, unlike some published studies, the present studies did not exclude equivocal patients or images in which myocardial regions were not observed or were of poor quality.[24,28–30]

The results of safety evaluation in RAMP-1 and -2 suggest that Imagify PSE is safe and well tolerated. Although the trial enrollment was completed prior to the updated warnings on contrast use, unstable patients were excluded from the trials and thus the study population complies with the current FDA recommendations.[31] No unifying mechanisms for the SAEs were apparent; SAEs appeared to have been isolated events that did not suggest any clinical trends. Most AEs reported were mild, transient, resolved without residual effects, and did not require treatment. Headache, chest pain, flushing, nausea, and chest discomfort were the most frequently reported AEs across the RAMP trials, and mainly occurred following dipyridamole infusion. These AEs are similar to those reported for dipyridamole during registration trials of SPECT imaging.[32]

Clinical implications

Unlike SPECT, PSE can offer immediate results with images acquired and interpreted in real-time. Thus, PSE offers the potential for a comprehensive, radiation-free, and potentially less costly (based on current contrast agent and SE costs) diagnostic evaluation of the effects of CAD on cardiac structure, function, and perfusion. In addition, PSE in the setting of conduction abnormalities has been shown to be superior to SPECT imaging.[33]

RAMP-1 represents the majority of patients (low-intermediate CAD probability) typically referred for non-invasive testing. RAMP-2 patients (intermediate-high CAD probability) went to ANGIO or already had ANGIO but required functional testing to assess significance of coronary lesions. Together, RAMP-1 and -2 represent a typical clinical population who are referred for non-invasive testing in clinical cardiology and represent the intended treatment population for PSE. Moreover, patients were drawn from a variety of care settings including private, public, academic, and Veterans Administration hospitals from nine countries. Thus, the conclusions drawn from these trials should be widely applicable and provide evidence to support that Imagify PSE is a potential alternative to gated SPECT for the detection of CAD and for risk-stratification of patients who may require the more invasive procedure, ANGIO.

Study limitations

The PSE evaluations did not discriminate between perfusion and wall motion abnormalities. An additional ROC analysis performed on a subgroup of patients without prior history of AMI demonstrated similar performance between PSE and SPECT (AUC: 0.69 for PSE; AUC: 0.71 for SPECT) for detection of inducible defects, which were likely due to reversible perfusion abnormalities as the dipyridamole dose used in these studies infrequently causes WMA.[19–21] Because perfusion and wall motion were being assessed concurrently and abnormalities are often present simultaneously, it was impractical for the reader to objectively separate these two characteristics. Furthermore, the trial was not designed to examine wall motion separately from perfusion as would be required to establish the relative roles of these two components. Moreover, SPECT readers had gated imaging to review for wall motion and thickening, consistent with routine clinical practice for SPECT, which integrates the evaluation of perfusion and wall motion.

SPECT analyses myocardial perfusion quantitatively and wall motion qualitatively, whereas PSE analyses both functions qualitatively. Development of quantitative methods for analysing PSE images may improve diagnostic performance of PSE for CAD detection.[29,34–37]

Although CAD truth assessment in these trials was based primarily on quantitative coronary angiography, many patients in RAMP-1 did not have angiographic data and had CAD status defined, as stated previously. This heterogeneity of truth standard in RAMP-1 is considered a reasonable compromise in study design since it is anticipated that approximately half the patients who would use PSE imaging will not undergo ANGIO. Furthermore, PSE performance in the subpopulation of RAMP-1 patients who underwent ANGIO was comparable to performance observed in the whole population.

Sensitivity and specificity of PSE readers in RAMP-1 and -2 ranged from 50 to 78% and 55 to 88%, respectively, with mean inter-reader agreement of 73%. This agreement will likely improve as readers gain more experience in image interpretation and as qualitative and quantitative methods and standards evolve. Moreover, the estimates of inter-reader agreement observed among PSE readers were similar to those observed among RAMP-2 SPECT readers (78%). These are similar to levels of agreement reported for SPECT readers in other clinical trials.[38,39]

Conclusions

Imagify PSE is a safe, non-invasive method for assessing CAD. The findings from the two RAMP trials support that PSE with Imagify is clinically equivalent to gated ^{99m}Tc SPECT perfusion imaging for the detection of CAD in patients with stable chest pain being evaluated for inducible ischaemia.

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