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HISTORICAL PERSPECTIVE

Myocardial Contrast Echocardiography

A Wondrous Journey!

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There are only two mistakes one can make along the path to truth: not going all the way, and not starting.

—Buddha (1)

This was in 1982. I was a clinical fellow in the Wadsworth Veterans Administration Hospital, Los Angeles cardiovascular training program, when I was first exposed to myocardial contrast echocardiography (MCE). I was enthralled by images obtained in a dog by Chuwa Tei who was working at that time with Pravin Shah in Elliott Corday's and Sam Meerbaum's experimental laboratory at Cedars-Sinai. Shortly thereafter, I moved to Massachusetts General Hospital for a clinical and research fellowship. My training in Boston was in both nuclear cardiology and echocardiography under a cardiovascular imaging training (T-32) program that was probably the first of its kind. I even dabbled in magnetic resonance imaging, which was in its very early days. I chose to work on MCE as my major research project in echocardiography. The echocardiography section did not have its own experimental laboratory, and I worked in the laboratory of Rob Okada. I had never been in an experimental laboratory before. Between Rob's skilled technicians, Donna Lutrario and Luis Guerrero, I learned complex coronary surgery, including cannulation of the left main coronary artery, which was to become the mainstay of many future experiments. From Nat Pandian I learned how to image open-chest dogs, Ned Weyman tried his best to teach me how to write, and John Newell taught me biostatistics.

When I started doing these experiments, reports of early MCE by Armstrong et al. (2) and Tei et al. (3) had already started to appear showing the feasibility of using MCE for assessing myocardial perfusion. Andy Kemper then published some excellent reports while he was working with Al Parisi at the Roxbury Veterans Administration Hospital (4,5). The concepts in MCE were probably ahead of their time. My initial interest was in defining area at risk in vivo. At that time the experimental gold standard was post-mortem technetium autoradiography. It soon became clear that with MCE we could measure risk area noninvasively and repeatedly (6). It also became clear that the risk area was not static and dynamically changed based on systemic and coronary hemodynamic conditions, including the coronary driving pressure (7). Also, there was a poor relationship between risk area size and hemodynamics, particularly when risk area was small-to-moderate, suggesting that hemodynamics poorly reflected the extent of myocardial injury during acute coronary occlusion (8).

After completing my fellowship at Massachusetts General Hospital, I moved to the University of Virginia (UVA), Charlottesville as a junior faculty and was given an old ultrasound system (ATL Mark 3) in the corner of a communal experimental laboratory. Because of my background in nuclear cardiology, I decided to measure myocardial blood flow (MBF) with

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MCE. Until then I had used intracoronary injections of hand-agitated mixtures of Renografin and saline, and needed to learn to make microbubbles that were small enough to pass through the capillaries. Steve Feinstein, who had been a fellow with me in Los Angeles and had developed a method for making smaller bubbles by sonicating albumin solutions (9), came to UVA and taught us the technique. Mark Keller was the first fellow to work with me and studied the intravascular rheology of these bubbles in Brian Duling's laboratory. He demonstrated that these bubbles behaved like erythrocytes within the microcirculation (10). Ananda Jayaweera subsequently showed that the transit time of these microbubbles through the myocardium was similar to that of radiolabeled erythrocytes during intracoronary injections at various flow conditions (11). After Jonathan Lindner joined the laboratory, we observed that during noncritical stenosis when MBF remained normal, the myocardial transit times for a microbubble was prolonged in proportion to the severity of the stenosis because of the increase in coronary blood volume caused by autoregulation (12). Therefore, using intracoronary injections, we could define the presence and severity of coronary stenosis at rest by measuring the myocardial transit times of microbubbles. We were to revisit this novel finding a decade later.

By this time, we had also started performing human studies in the cardiac catheterization laboratory, and reported the safety of intracoronary administration of sonicated Renografin and the feasibility of detecting a stenosis in conjunction with coronary vasodilators. We defined the value of MCE-derived collateral flow in maintaining myocardial viability after myocardial infarction (MI) (13). This attracted attention from the cardiology community because it provided for the basis of better outcomes in patients with an open infarct-related artery in the presence of collateral-dependent viable tissue in the infarct zone. A decade later, Matt Coggins, a medical student, showed that collateral flow could be measured using intravenous administration of microbubbles and that the extent of collateralization at the time of coronary occlusion predicted the ultimate infarct size after reperfusion (14). Howard Leong-Poi went on to demonstrate that collateral flow was responsible for the disparity between the circumferential extent of regional dysfunction and infarct size and not tethering as previously believed (15). The collateral flow also explained the disparity between the circumferential extent of regional dysfunction and perfusion defect during stress, lending to the superiority of myocardial perfusion over regional function for detecting coronary artery disease (CAD) during stress (15). Howard was also the first to report on the occurrence of a perfusion defect before regional dysfunction during demand ischemia (16).

MCE gained further notoriety when Hiroshi Ito used it to define the no-reflow phenomenon in acute MI; he showed that a sizeable proportion of patients with Thrombolysis In Myocardial Infarction (TIMI) flow grade 3 after thrombolysis had no-reflow, and that the degree and extent of no-reflow defined outcome in these patients (17). At the same time, Michael Ragosta, a fellow working with us, reported that the extent of microvascular perfusion and not TIMI flow predicted functional recovery in recent MI with an open infarct-related artery (18). He won the American College of Cardiology Young Investigator Award for this work. Liza Villanueva performed a series of elegant canine experiments to show that the no-reflow phenomenon was dynamic for hours after reflow, mostly due to the hyperemic response during reperfusion (19). It is now becoming clear that the best time for estimation of the no-reflow zone is at least 48 h after reperfusion, and that a coronary vasodilator will need to be used if the no-reflow zone (and, hence, infarct size) is to be estimated earlier after the reperfusion. The hyperemia in the reperfused bed is less than the perfusion in the normal bed, and the relative hypoperfusion noted in the reperfused bed during exogenously induced hyperemia predicts the ultimate no-reflow zone (20). Interestingly, whereas the no-reflow zone at rest is dynamic for several hours after reperfusion, it remains constant during hyperemia and accurately reflects the ultimate infarct size (19).

At about the same time, we, along with Bill Spotnitz, had developed an interest in assessing myocardial perfusion intraoperatively during coronary artery bypass grafting (CABG). We validated the methodology in canine experiments (10) followed by clinical studies in patients undergoing CABG (21,22). Liza also devised complex experiments to define the mechanism of myocardial preservation during retrograde cardioplegia delivery (23). Despite its obvious advantages in patients undergoing CABG, MCE using this approach was not widely adopted because imaging was to be performed with a hand-held transducer and subsequently due to the arrival of more versatile transesophageal echocardiographic technology.

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Steve Feinstein's technique of sonication was adapted for the first commercially available ultrasound contrast agent, Albunex, for use as an intravenous agent for left ventricular (LV) cavity opacification (8). Although this agent produced excellent myocardial opacification on intracoronary injections, it barely produced LV cavity opacification after intravenous administration. It became clear then that the air in the bubble diffused out and dissolved rapidly in blood, necessitating a new generation of ultrasound contrast agents. The newer agents either contained high molecular weight gases that were not easily diffusible and relatively insoluble in blood (such as sulfur hexafluoride, perfluorocarbons), or were made of impermeable shells (such as polymers). These agents are now in clinical use, and several investigators, such as Paul Grayburn, have demonstrated the value of LV cavity opacification for improving image quality and accuracy of LV ejection fraction measurements (24). Paul and others have also demonstrated the feasibility of microbubble-based gene delivery (25).

Despite the new contrast agents, myocardial opacification after intravenous injection had not been perfected for the following reasons. First, it is usually difficult to optimally differentiate the bubble signal from that of the myocardium, which has been partially solved by the development of unique signal processing algorithms, such as harmonic imaging. Because the bubbles resonate in an ultrasound field, they were induced to generate harmonic signals that could then be captured using a broad-band transducer. At that time it was believed that tissue, being noncompressible, did not generate harmonics. So it came as a surprise when superior tissue images were obtained using this approach. Harmonic imaging has now become the standard approach for obtaining B-mode ultrasound images, and this technological advance is a byproduct of MCE research. However, because tissue was still visible on harmonic imaging, we developed off-line image processing tools to detect myocardial opacification by the microbubbles (26). Jiri Sklenar, a computer scientist, is credited for developing algorithms for image alignment, subtraction, color-coding, and display for accurate delineation of myocardial opacification. This process, although very effective, was tedious before the days of digital echocardiography and involved many steps including digital conversion of analog data. It was later superseded by clever signal processing algorithms that exploited the nonlinear signals emanating from microbubbles oscillating in an ultrasound field and negating those arising from the tissue. These advances became possible by the fundamental discoveries of Peter Burns (27) and Nico de Jong (28), who characterized the microbubble-ultrasound interactions. Using digital image processing, Villanueva was the first to demonstrate LV myocardial opacification in dogs using right-sided injection of concentrated Albunex (26).

The second reason for poor myocardial opacification was the destruction of microbubbles with ultrasound. This became apparent when Tom Porter accidentally stopped ultrasound transmission during a canine experiment at the University of Nebraska (29). On resuming ultrasound, he noted excellent myocardial opacification on the first few frames before myocardial opacification again disappeared. Until then, imaging had been performed using relatively higher power, and the oscillating bubbles were imploding from the ultrasound energy. It became apparent that decreasing the power output would allow better LV opacification. Together with the newer signal processing algorithms, myocardial opacification could be detected even with lower power output. Tom Porter became interested in MCE while he was a fellow at the Medical College of Virginia and visited UVA to discuss his projects and results with me. In addition to becoming a leader in the field, he has also pioneered other areas of microbubble-ultrasound interactions such as sonothrombolysis (30) and drug/gene delivery (31).

With the development of the second-generation contrast agent containing a high molecular weight gas, Optison, and harmonic imaging, we were eager to demonstrate myocardial opacification in humans from intravenously administered microbubbles. However, since at that time both the ultrasound contrast agent and the imaging modality were 'experimental', we were not able to do so in the U.S. In collaboration with Roxy Senior at Northwick Park Hospital, London, we studied 30 patients within a week who were originally scheduled for dipyridamole sestamibi-single-photon emission computed tomography (SPECT) myocardial perfusion imaging. There was a good concordance between SPECT and image-processed color-coded MCE images for reversible and fixed defects, and showed the feasibility of the clinical use of intravenously administered ultrasound contrast agent for the detection of CAD (32). This was Roxy's first encounter with MCE, and he has since become a leading exponent of this discipline. He has worked extensively on the value of MCE in the clinical assessment of myocardial viability.

Since individual MCE images represent a single point in time, myocardial acoustic intensity values from these images reflect myocardial blood volume (MBV) and not MBF. With the knowledge that ultrasound can be used to destroy microbubbles, we developed an approach for the quantification of MBF that measured both components of MBF-MBV and MBF velocity (33). At that time Kevin Wei had joined my laboratory, and he worked extensively at validating microbubble destruction and replenishment method for MBF determination, both in experimental and clinical studies. Ananda Jayaweera developed a simple and elegant mathematical model for tissue nutrient flow measurement which has become the standard for tissue perfusion measurement in the heart, brain, kidney, skin, and skeletal muscle. Kevin was awarded the best Young Investigator Award at the American College of Cardiology for this work. Jiri Sklenar complemented by developing nimble computer algorithms for image analysis that allowed for the quantification of tissue flow. Using this approach, Andre Linka demonstrated that subendocardial ischemia in the presence of critical coronary stenosis was due to reduction in endocardial blood flow velocity and not MBV (34).

Once the MBF-flow velocity and MBV could be differentiated, it became possible to identify the reversible perfusion defects that we had originally noted to be due to a decrease in MBV during vasodilator stress. Using a novel model, Jayaweera demonstrated that in order to maintain a constant hydrostatic pressure, capillaries distal to a noncritical stenosis derecruit during pharmacological stress, which forms the basis for the reversible decrease in MBV and the reversible perfusion defect seen on any form of myocardial perfusion imaging where a tracer is used (35); this was subsequently confirmed for ^{99m}technetium sestamibi as well (36). Jayaweera demonstrated that the zero flow coronary pressure thought commonly to result from collateral flow occurred from the collapse of capillaries as perfusion pressure fell during reduced resting flow. As such, the bottleneck to hyperemic flow was the capillary bed. Because capillaries are placed in parallel, less capillaries result in lower flow reserve such as in MI and hypertension. Elizabeth Le later suggested that the capillaries regulate their hydrostatic pressure by derecruiting when autoregulation is exhausted (37).

In an ischemia-reperfusion model of constant coronary flow and microbubble administration directly into the coronary arteries, we observed that after transiting through the myocardium within a few seconds at baseline, a proportion of microbubbles remained in the tissue resulting in persistent myocardial opacification after reperfusion (38). The same phenomenon was seen in patients in the operating room after bypass surgery after cold crystalline solution compared with warm blood cardioplegia (39). Jonathan Lindner investigated this phenomenon in Klaus Ley's laboratory with intravital microscopy using an exteriorized microcirculation preparation. The microbubbles adhered nonspecifically to the adhesion molecules expressed on leukocytes and endothelial surface of venules during ischemia and reperfusion (40). This also demonstrated the feasibility of molecular imaging employing microbubbles and ultrasound. Jonathan and Liza have conducted numerous studies in inflammation and angiogenesis using microbubbles targeted to the endothelial surface. Because of the nonlinear signals emanating from microbubbles, very few need to be present within tissue in order to produce adequate signal. Jonathan has also studied skeletal muscle microcirculation with microbubbles in animal models and in patients presenting with peripheral vascular disease (41,42).

Based on the original demonstration that coronary blood volume increased in proportion to the coronary severity as long as coronary flow was constant (noncritical stenosis), we have always been intrigued by the possibility of using ultrasound contrast to detect CAD at rest. Because the blood in intramyocardial arterioles comprised only a small proportion of MBV, microbubble signals from these vessels were normally negligible when the ultrasound beam was fully replenished. However, if imaging was performed using a very short interval between destructive ultrasound pulses, the signal obtained was derived only from the vessels that filled in the short period of time, as neither capillaries nor venules had adequate opportunity to fill. Since forward flow in large intramyocardial vessels occurs during diastole, signals on MCE using this approach were seen predominantly during diastole. During systole, a change in myocardial elastance caused retrograde displacement of the arteriolar blood volume into large intramyocardial vessels, resulting in a small systolic signal on MCE. In the presence of a stenosis, since arteriolar blood was larger because of autoregulation, there was greater retrograde displacement of microbubbles from smaller arterioles and an increase in the systolic myocardial signal from the large intramyocardial vessels. Because these vessels do not participate in autoregulation, the diastolic signal remains unchanged, and the ratio of systolic to diastolic signal

increased with more severe stenosis. Wei performed painstaking experiments to validate this approach for CAD detection at rest without the use of any stressors in both animals (43) and humans (44).

Since capillaries are the bottleneck to hyperemic flow, factors influencing capillary resistance (mainly viscosity) could affect hyperemic flow. Se-Joong Rim showed that hyperlipidemia reduced flow reserve by increasing myocardial vascular resistance through an increase in whole blood viscosity (45). Jian-Ping Bin showed that nitroglycerin increased microvascular flow in the ischemic bed by reducing blood viscosity (46), and also induced hemoglobin to unload more oxygen in hypoxic tissue. Alcohol did not change rheology or increase coronary flow reserve as reported previously.

With the advent of many second- and third-generation contrast agents (Optison, Definity, Sonovue, Imagent, Sonozoid, Cardiosphere, Imagify), several validation studies were performed in experimental studies as well as phase II and III clinical trials. These studies have showed equivalence or even superiority of MCE to radionuclide myocardial perfusion imaging during pharmacological stress in patients with suspected or known CAD (47–49). MCE was equivalent to SPECT imaging in detecting acute coronary syndromes in patients presenting to the emergency department with chest pain (50); MCE imaging demonstrated incremental value (51,52) and was superior to the TIMI score at the time of initial emergency department presentation (53).

The reason this journey is termed as wondrous is that this field constantly keeps one in awe. When I first injected microbubbles into the coronary arteries of dogs at Mass General, I had no idea where the journey would lead. It was not even certain whether microbubbles would ever be able to cross the lungs. This journey has taught the intricacies of ultrasound physics, microbubble engineering, and the complex and fascinating physiology of the coronary microcirculation. It has helped design clinical trials, and exposed investigators to the procedures and policies of the Food and Drug Administration, Centers for Medicare and Medicaid Services, and the National Institutes of Health. It has taught how difficult and expensive it is for start-up companies to establish a new product, and how reluctant physicians are to adopt a new technology.

The MCE journey has allowed an excellent combination of basic and clinical sciences. I have been fortunate to be at the center-stage of MCE research supported by outstanding colleagues. A significantly large volume of literature has been produced pertaining to basic science, physiology, pathophysiology, and potential clinical applications of contrast-enhanced echocardiography. What is left is to translate it into routine clinical practice; this next task ahead of us is perhaps the hardest to achieve. Nonetheless, this journey has been immensely fulfilling; to quote the Buddha, "It is better to travel well than to arrive" (54).

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