Noninvasive Quantification of Coronary Blood Flow Reserve in Humans Using Myocardial Contrast Echocardiography

Kevin Wei, MD; Michael Ragosta, MD; Jerrel Thorpe, RDCS; Matthew Coggins, BA; Sally Moos, RDCS; Sanjiv Kaul, MD

Background—We hypothesized that coronary blood flow (CBF) reserve could be quantified noninvasively in humans using myocardial contrast echocardiography (MCE).

Methods and Results—Eleven patients with normal epicardial coronary arteries (group I) and 19 with single-vessel coronary stenosis (group II) underwent quantitative coronary angiography, MCE, and CBF velocity measurements at rest and during intravenous adenosine infusion. In group I patients, MCE-derived myocardial blood flow (MBF) velocity reserve (2.4±0.08) was similar to CBF velocity reserve using a Doppler flow wire (2.4±1.1). Patients with a single risk factor had a significantly higher MBF reserve (3.0±0.89) than those with ≥2 risk factors (1.7±0.22). In group II patients, significant differences were found in MBF velocity reserve in patients with mild (<50%), moderate (50% to 75%), or severe (>75%) stenoses (2.2±0.40, 1.6±0.65, and 0.55±0.19, respectively; P=0.005). A linear relation was found between flow velocity reserve determined using the 2 methods (r=0.76, P<0.001), and a curvilinear relation was noted between the percent coronary stenosis measured using quantitative coronary angiography and velocity reserve using both methods.

Conclusions—CBF reserve can be measured in humans using MCE. This method may allow the noninvasive assessment of coronary stenosis severity and the detection of microvascular dysfunction. (Circulation. 2001;103:2560-2565.)

Key Words: blood flow ■ coronary disease ■ contrast media ■ echocardiography

Because the microvascular rheology of the microbubbles used for myocardial contrast echocardiography (MCE) is similar to that of red blood cells, MCE has been shown to noninvasively and accurately quantify myocardial blood flow (MBF) velocity. In this study, we hypothesized that MCE could also be used to determine coronary blood flow (CBF) velocity reserve noninvasively in humans by measuring microbubble (or red blood cell) velocity at rest and during maximal hyperemia. This hypothesis was tested in patients undergoing cardiac catheterization. MCE data were compared with CBF velocity measurements using an intracoronary Doppler flow wire, and both sets of data were compared with percent luminal diameter stenosis from quantitative coronary angiography (QCA).

Methods

Patient Population

The study protocol was approved by the Human Investigation Committee at the University of Virginia. A total of 30 patients >18 years of age who were scheduled for elective coronary angiography were enrolled in the study. Group I patients (n=11) had angiographically normal coronary arteries, and group II patients (n=19) had a noncritical coronary stenosis in a single vascular territory. Patients were also screened to evaluate their echocardiographic image quality. Those in whom both the endocardial and epicardial borders in the coronary artery territory of interest could not be visualized were not recruited. All patients gave written, informed consent to participate in the study.

Myocardial Contrast Echocardiography

Intermittent harmonic imaging was performed with a phased-array system interfaced to a S3 transducer that transmits ultrasound at a mean frequency of 1.3 MHz and receives it at a mean frequency of 3.6 MHz. The transmit power was set at maximum, and compression was set at 50 dB. Gain settings were optimized at the beginning of each study and subsequently held constant. Imaging was performed using either the apical 4-, 2- or 3-chamber views, depending on the perfusion territory of interest, which was based on the angiographic results. The pulsing interval (PI) was gated to the ECG and progressively increased from 80 ms to 10 s. Up to 8 images, acquired at each PI, were recorded on 1.25-cm S-VHS videotape (Panasonic AG-MD830, Matsushita Electric Corp).

MCE was performed using a continuous infusion of Definity. A dose of 0.04 mL/kg was diluted in 100 mL of normal saline and infused intravenously at a rate of 120 to 180 mL/h. The adequacy of myocardial opacification was confirmed by imaging at every fifth cardiac cycle, and the infusion rate was individually adjusted to produce adequate myocardial opacification with minimal left atrial shadowing. Absence of any change in myocardial video intensity (VI) over 3 successive frames by visual assessment indi-
cated that steady state had been reached (~2 minutes after starting the infusion).3

Data were transferred from videotape to an off-line computer for analysis.3 At least 5 images acquired at baseline (precontrast) and at each PI were manually aligned. Only images in the same location within the ultrasound sector and with a similar orientation were selected. Those that were either shifted due to breathing or were off-axis due to changes in transducer position or patient movement were not selected for alignment. Large regions of interest were placed over the mid-myocardium, and VI was automatically measured from these regions from each of the aligned images. PI versus background-subtracted VI plots were then generated, and they were fitted to the exponential function \( y = A(1 - e^{-t}) \), where \( y \) is the VI at a PI of \( t \), \( A \) is the plateau where VI represents myocardial blood volume, and \( \beta \) represents the mean microbubble velocity.3

In addition to the above analyses, color-coding was applied to background-subtracted images to visually enhance regional differences in myocardial contrast enhancement.3 All pixels with a gray-scale value >10 were assigned a color based on the degree of contrast enhancement, where shades of red, progressing to hues of orange, yellow, and white represent incremental contrast opacification. Pixels with gray-scale values ≤10 were considered to represent noise and were not assigned a color. The left ventricular cavity was masked out.

QCA and Determination of CBF Velocity

All QCA and Doppler flow wire measurements were performed by a single experienced operator (M.R.) who has performed >100 of these procedures. A 0.014-inch intracoronary Doppler flow wire (FloWire, Cardiometrics) was placed in the vessel of interest.4 In group I patients, the wire was placed in a nonbranching portion of the mid-left anterior descending (LAD) coronary artery. Care was taken to position the tip of the wire in the middle of the lumen, as coaxial as possible with the walls of the vessel, and in a location that gave the best spectral Doppler signal. The position of the tip of the flow wire was documented by cineangiography. The average peak velocity was automatically determined by integration of the spectral Doppler signal.4 In group II patients, spectral Doppler signals were continuously recorded as the flow wire was advanced into the coronary artery. Baseline flow velocities proximal to the stenosis were first determined. As the wire was advanced across the lesion, flow velocities increased, and the wire was continually advanced to a location where all poststenotic acceleration of flow had ceased (beyond the vena contracta).

For QCA, digital cineangiographic images acquired in orthogonal projections were used. Calibration was performed using the coronary guiding catheter as a reference (6F=2.0 mm, 7F=2.75 mm, and 8F=2.67 mm), with the image magnified 4×. The coronary artery segment of interest was selected, and the axis and edges of the artery were automatically defined. In group II patients, minimal lumen diameter and percent diameter stenosis were computed in a standard manner using the proximal normal vessel segment as the reference in a single-plane, “worst-view” angulation.6 The automated edge detection methodology was visually inspected to assure that lumen edges were correctly identified.

Protocol

Coronary angiography was first performed to define the coronary anatomy. CBF velocity and hemodynamics were acquired, and QCA and MCE were performed at baseline. An intravenous infusion of 140 \( \mu \)g · kg⁻¹ · min⁻¹ adenosine (Adenoscan, Fujisawa Healthcare, Deerfield, Ill) was then initiated to induce maximal coronary hyperemia. CBF velocity was measured along with other hemodynamics, and QCA and MCE were repeated. If high-dose adenosine could not be tolerated by the patient (because of hypotension, adverse effects, etc), the dose was reduced to 70 \( \mu \)g · kg⁻¹ · min⁻¹. CBF velocity and MBF velocity reserve were calculated by dividing the values obtained during maximal hyperemia by those obtained at rest.

Statistical Methods

Comparisons between 2 stages were made with unpaired Student’s \( t \) test, and those between >2 stages were performed with repeated measures ANOVA. Interstage differences were confirmed using an unpaired Student’s \( t \) test. All correlations were performed with least-squares fit regression analysis. Differences were considered significant at \( P<0.05 \) (2-sided).

Results

Group I Patients

MCE data were not suitable for analysis in one group I patient because of image artifacts, so data from the remaining 10 patients are presented. Their median age was 47 years (range, 37 to 61 years), and 6 patients were men. By design, none of the patients had any coronary artery stenoses. Background-subtracted color-coded images (apical 2-chamber view) from one patient at a short PI from baseline and during adenosine infusion are illustrated in Figure 1. The corresponding Doppler flow wire velocity signals from the 2 stages are also shown. The average peak velocity increased from 28 to 89 cm/s during adenosine infusion. At baseline, little myocardial contrast enhancement is seen at the short PI (1.8 s) shown. The increase in CBF velocity during adenosine resulted in more rapid microbubble replenishment of the myocardial microcirculation, causing a greater degree of myocardial contrast enhancement at a PI of 1.5 s compared with baseline.

The PI versus VI curves obtained at baseline and during hyperemia from the patient in Figure 1 are shown in Figure 2. The microbubble velocity is more rapid during adenosine infusion than at baseline. In this instance, \( \beta \) increased from 0.5 s⁻¹ to 0.9 s⁻¹ during adenosine, which matched the increase in average peak velocity on Doppler flow wire. The plateau VI (myocardial blood volume), however, was similar during the 2 stages, because myocardial blood volume did not change in the absence of any changes in systemic hemodynamics during adenosine.3

Figure 1. Background-subtracted color-coded images from an apical 2-chamber view from a group I patient with normal coronary arteries at baseline (top) and during adenosine infusion (bottom). The corresponding Doppler flow wire velocity signals from the 2 stages are shown on the right. See text for details.
Table 1. Hemodynamic, Doppler Flow Wire, and MCE Data From Group I Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Hyperemia</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>71±10</td>
<td>73±12</td>
<td>0.70</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>84±12</td>
<td>83±8</td>
<td>0.70</td>
</tr>
<tr>
<td>APV, cm/s</td>
<td>20±10</td>
<td>44±22</td>
<td>0.003</td>
</tr>
<tr>
<td>Microbubble velocity, s⁻¹</td>
<td>0.6±0.2</td>
<td>1.4±0.7</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are mean ± SD. MAP indicates mean arterial pressure; APV, Doppler flow wire–derived average peak velocity.

Figure 2. The PI versus VI curves obtained from the LAD bed of patient whose images are shown in Figure 1. The results from baseline (○) and hyperemia (●) are shown. See text for details.

The hemodynamic, Doppler flow wire, and MCE data acquired at baseline and during adenosine infusion from all group I patients are shown in Table 1. The mean dose of adenosine used was 95±35 μg · kg⁻¹ · min⁻¹. Doppler flow wire average peak velocity more than doubled, and the epicardial CBF almost tripled, resulting in a CBF velocity reserve of almost 2.5 and a CBF reserve of nearly 3.0. 

In the absence of any changes in systemic hemodynamics during adenosine, no significant change in MBV or plateau occurred at baseline and hyperemic stages (26±11 versus 30±8, respectively, P=0.30). The increase in MBF during adenosine was therefore met entirely by increases in microbubble velocity (Table 1). The microbubble velocity reserve determined using MCE was identical to CBF velocity reserve determined from Doppler flow wire. There were 5 group I patients with only a single cardiac risk factor (2 with hypertension and 3 with hypercholesterolemia, all of whom were on medical therapy); the other patients had ≥2 risk factors. Both the MBF and CBF velocity reserve were significantly higher in patients with a single risk factor (3.0±0.89 and 3.7±1.1) than it was in those with ≥2 risk factors (1.7±0.2, P=0.01, and 1.6±0.4, P=0.002).

Group II Patients
MCE images could not be analyzed in 3 group II patients because of image artifacts, so data from the remaining 16 patients are presented. Their mean age was 56 years (range, 38 to 75 years), and 12 patients were men. Color-coded images of an apical 2-chamber view from a patient with a moderate LAD stenosis (60% by QCA) obtained at rest and during hyperemia are illustrated in Figure 3. The corresponding PI versus VI curves from the LAD and left circumflex beds are also shown. Because the stenosis was not flow-limiting at rest, the change in VI at different PIs is similar in both beds at baseline, resulting in a similar microbubble velocity. During hyperemia induced with 140 μg · kg⁻¹ · min⁻¹ adenosine, the increase in CBF velocity was less in the stenosed LAD compared with the left circumflex bed, resulting in lower microbubble velocities in the former. Therefore, at a short PI of 2 s, perfusion mismatch is noted on the MCE image. In this instance, peak VI was lower in the LAD bed, even at longer PIs.

The hemodynamic, Doppler flow wire, and MCE data acquired at rest and during adenosine infusion from all group II patients are depicted in Table 2. Stenoses in individual patients were divided into mild (<50%), moderate (50% to 75%), and severe (>75%) on the basis of QCA. No changes in heart rate or blood pressure were noted between rest and adenosine stages in any patient. None of the stenoses was critical in severity, so resting Doppler flow wire average peak velocities were similar between all patients. In contradistinction, during adenosine infusion, these values were significantly different between the 3 groups (Table 2).

Similar to the flow wire data, no significant difference was found in the absolute values of MCE-determined MBF velocity at rest for patients with mild, moderate, or severe stenosis (Table 2). Although absolute MBF velocity was not significantly different between the 3 groups during adenosine infusion, MBF velocity reserve was again significantly different between them (Table 2). Myocardial blood volume was similar between the 3 groups at rest (51±16, 52±18, and 56±0.10 for mild, moderate, and severe stenoses, respectively; P=0.94) and during hyperemia (48±15, 54±24, and 40±23 for mild, moderate, and severe stenoses, respectively; P=0.66).

β Reserve and CBF Velocity Reserve Versus Coronary Stenosis Severity
A linear correlation (y=0.6x+1) was noted between Doppler flow wire (x) and MCE-derived β reserves (y) from all patients (r=0.78, P<0.001, SEE=0.55). Figure 4 illustrates the relation between percent luminal diameter narrowing derived using QCA versus Doppler flow wire average peak velocity and MCE-derived MBF velocity reserve in group II patients. Both relations show a fit to the function y=A(1−e^(−bx)), but the fit for MCE-derived velocity is better. A failure of MBF velocity to increase >1.5 times that at baseline indicates the presence of >70% coronary artery stenosis.

Discussion
Measuring CBF Reserve with MCE
In this study, we showed for the first time that CBF reserve can be measured noninvasively in humans using MCE. We also showed that increases in microbubble (or red blood cell) velocity occur in parallel with increases in CBF induced by the administration of adenosine. Consequently, the ratio of hyperemic and resting MBF velocities measured with MCE correlates very well with CBF reserve.

Using a canine experimental model, we previously showed that during hyperemia, when coronary arterioles and venules are fully dilated, the resistance to CBF is mediated largely through capillaries,6 which lack smooth muscle and do not
change their size with a coronary vasodilator. When hyperemia is induced in the presence of a stenosis, myocardial vascular resistance distal to a stenosis increases, despite maximal dilatation of the coronary arterioles and venules.\textsuperscript{6,7} This increase in resistance is caused by a decrease in capillary blood volume distal to a stenosis, resulting in a reversible perfusion defect during myocardial perfusion imaging. We also showed that the magnitude of a perfusion defect during hyperemia is directly related to the severity of the stenosis.\textsuperscript{6,7} Thus, measuring plateau myocardial VI before and after the infusion of a coronary vasodilator also provides an assessment of the severity of coronary stenosis.

Although this method is accurate for the assessment of coronary stenosis severity in open-chest canine models, it has drawbacks in the clinical setting. First, the blood concentration of microbubbles must be the same during rest and hyperemia, which is not always feasible, even with a continuous infusion. We previously showed that although plateau VI was affected by small changes in the blood concentration of microbubbles, microbubble velocity was not similarly affected.\textsuperscript{1} Second, because the ultrasound backscatter within a myocardial region has to be compared between rest and stress stages, the imaging plane has to be identical for both examinations. Although this is possible in animal preparations, where the ultrasound transducer is held in a fixed position, it is difficult in patients. Third, the acoustic energy within the ultrasound field is heterogeneous, with less energy at the margins and at greater imaging depths.\textsuperscript{8} Consequently, the backscatter from microbubbles is also inhomogeneous. Attenuation-induced and other artifacts are also likely to cause a spurious decrease in backscatter. Although absolute backscatter estimates may be erroneous as a result of these

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stenosis Severity</th>
<th>Mild (&lt;50%; n=6)</th>
<th>Moderate (50–75%; n=7)</th>
<th>Severe (&gt;75%; n=3)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stenosis, %</td>
<td></td>
<td>34±11</td>
<td>62±7</td>
<td>79±5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR (BL), bpm</td>
<td></td>
<td>61±11</td>
<td>64±14</td>
<td>72±21</td>
<td>0.50</td>
</tr>
<tr>
<td>HR (AD), bpm</td>
<td></td>
<td>72±14</td>
<td>68±7</td>
<td>67±9</td>
<td>0.70</td>
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<tr>
<td>MAP (BL), mm Hg</td>
<td></td>
<td>105±17</td>
<td>91±22</td>
<td>100±37</td>
<td>0.51</td>
</tr>
<tr>
<td>MAP (AD), mm Hg</td>
<td></td>
<td>90±7</td>
<td>82±7</td>
<td>98±27</td>
<td>0.14</td>
</tr>
<tr>
<td>APV (BL), cm/s</td>
<td></td>
<td>21±9</td>
<td>31±18</td>
<td>19±6</td>
<td>0.36</td>
</tr>
<tr>
<td>APV (AD), cm/s</td>
<td></td>
<td>47±14</td>
<td>35±17</td>
<td>15±6</td>
<td>0.02</td>
</tr>
<tr>
<td>APV reserve</td>
<td></td>
<td>2.4±0.82</td>
<td>1.3±0.46</td>
<td>0.68±0.35</td>
<td>0.002</td>
</tr>
<tr>
<td>β (BL), s\textsuperscript{-1}</td>
<td></td>
<td>0.52±0.25</td>
<td>0.59±0.23</td>
<td>0.52±0.25</td>
<td>0.62</td>
</tr>
<tr>
<td>β (AD), s\textsuperscript{-1}</td>
<td></td>
<td>1.1±0.60</td>
<td>0.93±0.53</td>
<td>0.27±0.04</td>
<td>0.18</td>
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<tr>
<td>β Reserve</td>
<td></td>
<td>2.2±0.40</td>
<td>1.6±0.65</td>
<td>0.55±0.19</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Values are mean±SD. BL indicates baseline; AD, adenosine; HR, heart rate; MAP, mean aortic pressure; APV, Doppler flow wire-derived average peak velocity; and β, mean microbubble velocity.
factors, the rate of change of backscatter with increasing PIs does not change with adenosine in the absence of coronary stenosis,3 CBF reserve cannot be determined from measurements of plateau VI alone. Thus, as shown from the results of the current study, measuring changes in MBF velocity rather than plateau VI provides a more robust and accurate estimate of CBF reserve with MCE in the clinical setting.

CBF reserve measurements in the catheterization laboratory require expertise in both the Doppler flow wire technique and QCA. Proper use of the Doppler flow wire requires careful positioning of the wire tip in a fairly straight segment of the artery away from side branches. Highly tortuous and distal segments are difficult to assess with this technique. It is also necessary for the operator to recognize an acceptable Doppler signal and to interpret the data correctly both at baseline and under maximal hyperemia. QCA requires technical expertise as well. The lesion must be well opacified to allow accurate edge delineation, and the arterial segment must lie in the same plane as the catheter tip to allow proper calibration. The presence of inexperienced operators with either of these techniques might lead to inaccurate measurement of CBF reserve.

An interesting finding of our study is the curvilinear relation between MCE-determined MBF velocity reserve and percent coronary artery stenosis determined using QCA. This relation is more similar to that reported by Gould and Lipscomb9 than the relation between Doppler flow wire–determined CBF velocity reserve versus coronary stenosis (Figure 4). It is likely that in the latter situation, CBF velocity measurements were more prone to error from the angle dependence of Doppler than the measurement of tissue MBF velocity using MCE.

**Limitations**
Maximal doses of adenosine could not be used in all patients because of side effects. A higher dose of adenosine may have allowed greater increases in hyperemic CBF, which could potentially have permitted better separation of the degrees of stenosis and allowed the detection of milder stenoses. As would be expected, MBF and CBF velocity reserves of group I patients who had ≥2 risk factors were low in our study. Although these patients had normal coronary arteries on angiography, they were not evaluated by intravascular ultrasound or for the presence of microvascular disease. Our values of MBF velocity reserve, therefore, reflect the combined effects of both the epicardial coronary stenosis and microvascular dysfunction on flow reserve. Because the prevalence of these conditions was similar in our patients with different degrees of coronary stenosis, our data indicate that an abnormal MBF velocity reserve can still be used to detect physiologically significant stenoses.

The addition of subjects with a low probability of coronary artery disease would have been useful because they would most likely have shown an even higher reserve. The ability of MCE to measure high flows still needs to be demonstrated in humans, although it has clearly been shown in animals.3

Acquisition of MCE data are currently tedious and time-consuming. Image alignment, background subtraction, and selection of proper regions of interest require sufficient expertise and custom-designed software. Newer imaging methods are being developed that allow microbubble destruction with a high mechanical index followed by data acquisition using a low mechanical index that minimizes bubble destruction.10 Thus, PI versus VI curves can potentially be generated in real-time.

Although some bioeffects have been reported with the use of microbubbles and high-energy ultrasound, these have been noted only in ex vivo models where exposed muscle has been subjected to ultrasound exposure.11 Otherwise, microbubbles have been found to be safe in several thousand patients evaluated in clinical trials. As stated above, newer methods are currently being designed to image microbubbles in vivo using low-energy ultrasound.10

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References
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