Assessment of coronary microcirculation in a swine animal model

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1Department of Radiological Sciences, University of California-Irvine, Irvine, California; 2Department of Radiology, Tianjin Medical University General Hospital, Tianjin, China; and 3Department of Medicine (Cardiology), University of California-Irvine, Irvine, California

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Zhang Z, Takarada S, Molloi S. Assessment of coronary microcirculation in a swine animal model. Am J Physiol Heart Circ Physiol 301: H402–H408, 2011. First published May 27, 2011; doi:10.1152/ajpheart.00213.2011.—Coronary microvascular dysfunction has important prognostic implications. Several hemodynamic indexes, such as coronary flow reserve (CFR), microvascular resistance, and zero-flow pressure (Pzf), were used to establish the most reliable index to assess coronary microcirculation. Fifteen swine were instrumented with a flow probe, and a pressure wire was advanced into the distal left anterior descending artery. Adenosine was used to produce maximum hyperemia. Microspheres were used to create stenosis model (S model). The areas under the receiver operating characteristic curves were generated: normal epicardial artery model (N model) and stenosis model (S model). The areas under the receiver operating characteristic curves for flow probe-based CFR, angiographic CFR, NMRqh, NMRah, and Pzf in detecting the microvascular deterioration. Compared with CFR and Pzf, NMR provided a more accurate assessment of microcirculation. This improved accuracy was more prevalent when stenosis existed. Moreover, NMRah is potentially a less invasive method for assessing coronary microcirculation.

angiography; blood flow; cardiovascular imaging; coronary microvascular function

CORONARY ANGIOGRAPHY. A routine examination for patients with heart disease, provides an assessment of stenosis severity by visualizing the opacified arterial lumen. However, in many cases (12, 40), the severity of myocardial ischemia correlates poorly with epicardial stenosis. Therefore, intracoronary physiological techniques have been developed to provide physiological evaluations for patients who undergo coronary angiography (8, 34). Recently, the medical community’s interest in coronary microcirculation has grown with the increasing awareness that microvascular dysfunction occurs in a number of myocardial disease states and has important prognostic implications (19, 20). With this recognition comes the need for a method to accurately assess the functional capacity of coronary microcirculation for diagnostic purposes and to monitor the effects of therapeutic interventions targeted at reversing the extent of coronary microvascular dysfunction. To assess changes in the microcirculatory bed, several indexes have been proposed, such as coronary flow reserve (CFR), microvascular resistance (MR), and zero-flow pressure (Pzf). In the early 1990s, Gould et al. (14) introduced CFR as the first clinically applicable functional index for assessing epicardial vessel stenosis. It serves as a marker for the integrity of epicardial coronary circulation and microcirculation (36). MR is theoretically defined as the perfusion to coronary back pressure (vide infra) gradient, divided by absolute coronary blood flow at hyperemia (9). In 1974, Gould et al. (13, 15) postulated that the minimum MR is independent of epicardial stenosis severity. Pzf is the arterial pressure at which blood flow would cease. In 2003, Shimada et al. (31) speculated that Pzf increases with the severity of injured coronary microvasculature, particularly the capillaries (28). However, previous studies have not compared these indexes.

In a previous report, an angiographic technique for MR measurement, using a first-pass distribution analysis (FPA) technique in an in vivo coronary microvascular disruption model, was validated (43). The purpose of the present study is to compare the CFR, MR, and Pzf at various stages of severity in microvascular disruption and epicardial artery conditions by using both a flow probe as the gold standard and the angiographic method.

METHODS

Protocol. This study was conducted according to guidelines of the National Institutes of Health (NIH) and approved by the University of California, Irvine Institutional Animal Care and Use Committee. In an open-chest swine model, CFR, MR, and Pzf measurements were made at various stages of severity of microvascular disruption in the left anterior descending artery (LAD). Microcirculation was disrupted by embolized microspheres. An external vascular occluder was used to produce a moderate epicardial stenosis. Coronary angiograms were acquired for each data set.

Animal preparation. Fifteen fasted Yorkshire swine (37.6 ± 5.7 kg, male, S&S Farms) were sedated and premedicated with telazol-ketamine-xylazine (4.4, 2.2, 2.2 mg/kg, respectively) and atropine (0.05 mg/kg). Anesthesia was maintained with 1–2% isoflurane (Highland Medical Equipment Vaporizer, Temecula, CA). Carotid artery and jugular vein were surgically prepared for sheath placement. Adenosine (400 µg · kg⁻¹ · min⁻¹) (35) was used to induce maximum hyperemia.

Surgery and catheterization. A lateral thoracotomy was performed using standard surgical techniques. The fourth and fifth ribs were spread apart, and the heart was fully exposed. The pericardium was opened, and the proximal segment of the LAD was dissected free. A transit-time ultrasound flow probe (Transonic Systems, Ithaca, NY) was placed on the proximal segment of the LAD. An extravascular occluder (IVM In Vivo Metric, Healdsburg, CA) was placed around the LAD just distal to the occluder. An external vascular occluder was used to produce 100% occlusion for reactive maximum hyperemia. The position of the flow probe and the occluder was adjusted to ensure that there were no side branches between them. The occluder was also used to produce a 100% occlusion for reactive maximum hyperemia.
The left main ostium was cannulated with a 6F hockey stick catheter through the left carotid artery under fluoroscopic guidance. Another 4F hockey stick catheter was placed in the right atrium to measure the coronary back pressure. Microcirculation was disrupted by gradually injecting 50–100 μm (1.8 × 10^5) microspheres (Polysciences Warrington, PA) (2, 9) down the LAD through the catheter. This procedure was repeated for different degrees of severity of microcirculatory embolism. An intracoronary pressure wire (Radi Medical System, 0.14 in.) was advanced into the distal segment of the LAD to measure the distal coronary pressure (Pd). Aortic pressure (Pao), Pd, and right atrium pressure (Pv) were measured continuously with pressure transducers and the pressure wire, respectively.

**Image acquisition and processing.** All images were acquired using a conventional X-ray tube with a constant potential X-ray generator (Optimus M200, Philips Medical Systems, Shelton, CT). A cesium-iodide-based flat panel detector (PaxScan 4030A, Varian Medical, Palo Alto, CA) was used for image acquisition. Images were acquired at 30 frames/s. All images were corrected for X-ray scatter before logarithmic transformation (7). A publicly available software (Image J, NIH, Bethesda, MD) was used for image analysis.

Each swine was positioned on its right side under the flat panel detector. The projection angle was optimized for separating the LAD and the left circumflex artery perfusion beds. Pancuronium (0.1 mg/kg) was administered intravenously. Electrocardiogram, arterial blood pressure, and coronary blood flow were continuously recorded (MP100, Biopac Systems, Santa Barbara, CA) to establish that the animals were sufficiently sedated under this condition. Coronary angiograms were acquired when the blood flow reached maximum hyperemia. The ventilator was turned off at the end of a full expiration to minimize respiratory motion. When the blood flow reached maximum hyperemia, the angiography-based NMR (NMRab) was calculated as (Pd − Pao), divided by Qh during hyperemia. The angiography-based NMR (NMRab) was plotted with respect to the simultaneously measured Pao. The linear regression between Qh and Pao was used to calculate the slope of the curve in diastole, and the X-intercept of the line was used to calculate the Pao (Fig. 2). Every data point was calculated as the average of three consecutive cardiac cycles without recording artifacts.

**Comparison of hemodynamic indexes without epicardial stenosis.** CFRab, CFRq, NMRab, NMRqh, and Pzf were used to evaluate the different severities of coronary microvascular disruption. The measurements without epicardial stenoses were divided into four groups: group A (normal condition, N = 18), group B (mild microvascular disruption, <1.5 × 10^5 microspheres, N = 63), group C (moderate microvascular disruption, 1.5 × 10^5 to 3.0 × 10^5 microspheres, N = 51), and group D (severe microvascular disruption, >3.0 × 10^5 microspheres, N = 35).

**Diagnosis of microcirculation disruption involving epicardial stenosis.** Diagnostic abilities for microcirculation disruption using CFR, NMR, and Pzf were tested in two models: 1) normal epicardial artery model (N model), which included the normal condition and different severities of microvascular disruption with normal epicardial arteries; and 2) moderate epicardial stenosis model (S model), which included moderate coronary epicardial stenoses (50% diameter stenosis) and different severities of microvascular disruption with the same moderate epicardial stenoses. The gold standard for detecting microvascular disease is determined by whether microspheres were injected, regardless of the amount of microspheres.

**Statistical analysis.** Linear regression analysis was performed between 1) CFRab and CFRq, and 2) NMRab and NMRqh to determine the coefficient in the regression equation. The correlation coefficient (r) and standard error of estimate (SEE) were determined by a linear regression analysis. The degree of agreement between the different methods was assessed using the Bland-Altman analysis. One-way ANOVA and Student Newman-Keuls test were used to compare the CFRab, CFRq, NMRab, NMRqh, and Pzf in the four groups of different coronary microvascular conditions. Two series of receiver operating characteristic curves were made for both the N model and the S model. The areas under each curve (AUC) and the best cutoff values were calculated to compare the diagnostic abilities of the five dynamic indexes. A P < 0.05 was considered to be statistically significant for all statistical analyses.

**RESULTS**

From a total of 337 measurements, there were 167 measurements without epicardial stenosis and 170 measurements involving stenoses. Also, 251 measurements, excluding the conditions with only epicardial stenoses and without microspheres, were used to compare the angiographic and gold standard flow measurements.

**Comparison of angiographic and flow probe measurements.** A total of 251 CFR and NMR measurements were made. CFRab was linearly related to the gold standard CFRq as CFRq = 0.91 CFRab + 0.30 (P < 0.001) with a good correlation coefficient of 0.91.
NMRah correlated linearly with NMRqh as $NMR_{ah} = 0.89 \, NMR_{qh} + 0.04 \, \text{mmHg} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ ($P < 0.001$) with a good correlation coefficient ($r = 0.955$, SEE = 0.208 mmHg·ml$^{-1}$·min$^{-1}$). Additionally, in the Bland-Altman plot, the mean differences between the two measurements were 0.12 for CFR and 0.10 mmHg·ml$^{-1}$·min$^{-1}$ for NMR. The limits of agreement were 1.19 and 0.95 for CFR and 0.35 and 0.55 mmHg·ml$^{-1}$·min$^{-1}$ for NMR. None of the values had a statistically significant difference from zero, implying a lack of bias between the two techniques. Additionally, there were two observers measuring the angiographic flow using the FPA method for the 55 measurements taken from the three animals. The mean error for variability between two different observers was 4.95 ml/min (error relative to the mean was 7.9%), and the error for reproducibility was 4.83 ml/min (error relative to the mean was 7.7%).

Comparison of hemodynamic indexes without epicardial stenosis. Results from ANOVA showed significant differences ($P < 0.001$) among the four groups for each hemodynamic index in a total of 167 measurements. Figure 3 shows the Student Newman-Keuls multiple comparisons between the four groups for each index. For all of the indexes ($CFR_{q}$, $CFR_{a}$, $NMR_{qh}$, $NMR_{ah}$, and $P_{zf}$), there were significant differences between each group at various degrees of severity of microvascular disruption ($P < 0.05$).

Diagnosis of microcirculation disruption involving epicardial stenosis. In 167 measurements of the N model, the AUCs for $CFR_{q}$, $CFR_{a}$, $NMR_{qh}$, $NMR_{ah}$, and $P_{zf}$ were 0.855, 0.836, 0.976, 0.956, and 0.855, respectively. In 170 measurements of the S model, the AUCs were 0.737, 0.700, 0.935, 0.889, and 0.698, respectively (Fig. 4). There were significant area reductions for $CFR_{q}$, $CFR_{a}$, and $P_{zf}$ in the S model ($P < 0.05$), but no significant difference for $NMR_{qh}$ and $NMR_{ah}$ ($P > 0.05$). The percent area stenosis was measured to be $75 \pm 10\%$ (50% diameter stenosis). The $CFR_{q}$ was $2.82 \pm 1.17$ in the N model compared with $2.20 \pm 1.12$ in the S model ($P < 0.05$), and FFR was $0.74 \pm 0.14$ in the S model. Table 1 also shows the sensitivity and specificity of the best cutoff value for each index.

**DISCUSSION**

The present study demonstrated that NMR, which appeared to be independent of moderate epicardial artery disease, was the most reliable diagnostic index to detect microvascular
deterioration. Compared with CFR and Pzf, NMR showed improvement, especially in cases of moderate epicardial artery disease. Furthermore, the best cutoff value of NMR had the highest sensitivity and specificity compared with CFR and Pzf. Therefore, even in the presence of a moderate epicardial stenosis, the increase of NMR can predict coronary microvascular dysfunction.

Comparison of CFR, Pzf, and NMR. With the growing awareness that coronary microcirculatory dysfunction is an important pathophysiological component in many cardiac conditions, several different physiological dynamic indexes, such as CFR, Pzf, and NMR, have been introduced. From the present study’s results, CFR, Pzf, and NMR can all be used to evaluate the severities of microvascular disruption.

Some previous studies have used the concept of CFR as the theoretical framework to study microcirculation invasively (6, 23). CFR can provide an integral assessment of both epicardial coronary circulation and microcirculation. Quantitative assessment of CFR can be easily performed by intracoronary Doppler wires in the cardiac catheterization laboratory. However, the cutoff value for CFR is highly affected by epicardial stenosis and is very sensitive to hemodynamic changes (10, 29). Fractional flow reserve (FFR) has been established as a useful physiological index of the severity of an epicardial stenosis in the catheterization laboratory. FFR has previously been shown to be a reliable technique to functionally assess a given coronary intermediate stenosis with unclear hemodynamic significance (1, 5). A previous report has suggested that coronary outflow pressure-corrected FFR cannot only be considered a specific index for the epicardial stenosis alone (32). FFR is also a function of myocardial bed resistance, which varies as a consequence of variable hemodynamic conditions (32). Therefore, FFR cannot be used as an independent index to assess microcirculation, since it is highly affected by the presence of an epicardial stenosis. However, in the absence of microvascular disease, it is possible to estimate FFR using only angiographic image data (41, 42).

A previous study had reported that increased Pzf may reflect accentuation of microvascular tone and decreased perfusion bed mass, which is caused by nonviable tissue (8, 38, 39). Tanaka et al. (37) described this relationship during hyperemia. The slope of the curve in diastole and the X-intercept changed significantly during maximal hyperemia induced by papaverine administration. Ito (16) suggested that increased Pzf is responsible for the no-reflow phenomenon in patients. Pzf appears to be an accurate index for evaluating microcirculation, because only Q˙ and Pd are used during the diastolic phase to calculate Pzf. In diastole, coronary flow depends on coronary pressure without regard to cardiac contraction, and the diastolic relationship between coronary flow and pressure permits analysis of flow resistance.

Fig. 2. Zero-flow pressure (Pzf) measurement. The instantaneous blood flow (Q) from flow probe (Qp) was plotted against the simultaneously measured coronary distal pressure (Pd) displayed in an X-Y scatter plot. A: linear regression between Qp and Pd was used to calculate the slope of the curve in diastole, and the X-intercept of the slope was calculated as Pzf. B: the diastolic interval analyzed was selected from the maximal diastolic blood flow point to the beginning of the phase of rapid decrease of blood flow induced by ventricular contraction. Pa, aortic pressure.

Fig. 3. Comparison of hemodynamic indexes at different degrees of severity of microvascular disruption. Results from ANOVA showed significant differences (P < 0.001) in the four groups for each hemodynamic index in a total of 167 measurements. The Student Newman-Keuls multiple comparisons were made between the four groups for each index. For all of the indexes [gold standard flow probe-based coronary flow reserve (CFRg), angiography-based coronary flow reserve (CFR), flow probe-based normalized microvascular resistance (NMRp), angiographic normalized microvascular resistance (NMRa), and Pzf], there were significant differences between each group at different degrees of severity of microvascular disruption (P < 0.05). Values are means ± SD.
of coronary artery resistance (17, 28, 31). However, sometimes it is hard to perform $P_{zf}$ on patients using Doppler wires because of the large variance. $P_{zf}$ is easily influenced by individual cardiac cycle and diastolic phase.

Previous studies have reported that, compared with other indexes, MR’s advantages allow it to be reliably applied for the interrogation of microcirculatory resistance in the catheterization laboratory (4, 9, 18, 22). First, MR is reproducible and largely independent of variation in the hemodynamic state (29). Second, MR appears to be independent of epicardial artery disease. Previous reports (3, 30, 33) have documented an increase in MR in the presence of an epicardial artery stenosis. From the present study’s results, even in the presence of a moderate epicardial stenosis, the increase of NMR could still be used to evaluate the microvascular disruption. Additionally, MR is an independent predictor of acute and short-term myocardial damage in patients undergoing primary percutaneous coronary intervention. It may allow us to determine the efficacy of therapeutic strategies for microvascular protection in patients with ST-segment elevation myocardial infarction (11). Therefore, measurements of coronary MR play a pivotal role in diagnosing and monitoring the effects of therapeutic interventions. However, MR calculation requires an accurate measurement of absolute blood flow. A relatively simple and minimally invasive method for quantitatively assessing the status of the coronary microcirculation is still required.

**FPA and angiographic NMR.** Several invasive and noninvasive techniques have been developed for measuring blood flow to evaluate the status of coronary microcirculation (6, 18, 20). FPA technique, using angiographic image data, has previously been used to measure absolute coronary blood flow through a stenotic artery (26, 27). Our laboratory’s previous report (43) focused on validation of the FPA technique in a swine microvascular disruption model. It showed that angiographic NMR measurement based on the FPA technique has good accuracy and reproducibility. In the present study, different indexes for assessment of microvascular disruption were compared. Compared with CFR and $P_{zf}$, NMR was shown to be the most reliable diagnostic index to detect microvascular deterioration, especially in the presence of moderate epicardial artery stenosis. The results indicate that NMR can provide an accurate and minimally invasive assessment of coronary microcirculation in the catheterization laboratory. Furthermore, angiographic NMR based on the FPA technique has important advantages over the previously reported methods for MR measurement (24, 41). The angiographic NMR measurement, which requires no wires, reduces the cost and procedure time associated with the other techniques. All angiographic NMR measurements can be done using image data acquired during routine diagnostic cardiac catheterization. Therefore, both anatomic and physiological information can be derived from the same angiographic images. Therefore, the angiographic method for NMR measurement could potentially be used to

### Table 1. Diagnosis for microcirculation disruption

<table>
<thead>
<tr>
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<th>N Model ($N = 167$)</th>
<th>S Model ($N = 170$)</th>
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<tbody>
<tr>
<td><strong>AUC</strong></td>
<td><strong>Best cutoff value</strong></td>
<td><strong>Sensitivity</strong></td>
</tr>
<tr>
<td>$CFR_q$</td>
<td>0.855</td>
<td>3.389</td>
</tr>
<tr>
<td>$CFR_a$</td>
<td>0.836</td>
<td>3.760</td>
</tr>
<tr>
<td>$NMR_{ah}$, mmHg$\cdot$ml$^{-1}\cdot$min$^{-1}$</td>
<td>0.976</td>
<td>0.641</td>
</tr>
<tr>
<td>$NMR_{ah}$, mmHg$\cdot$ml$^{-1}\cdot$min$^{-1}$</td>
<td>0.956</td>
<td>0.632</td>
</tr>
<tr>
<td>$P_{zf}$, mmHg</td>
<td>0.855</td>
<td>26.563</td>
</tr>
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</table>

Areas under the receiver operating characteristic curves (AUC) and the best cutoff values of all the indexes in both normal (N) and stenotic (S) models. $CFR_q$, gold standard flow probe-based coronary flow reserve; $CFR_a$, angiography-based coronary flow reserve; $NMR_{ah}$, gold standard normalized microvascular resistance; $NMR_{ah}$, angiographic normalized microvascular resistance; $P_{zf}$, zero-flow pressure.

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![Fig. 4. Diagnosis for microcirculation disruption involving epicardial stenosis.](image-url)
evaluate the microcirculatory system of patients with stable chest pain in the cardiac catheterization laboratory.

The present study used \((P_d - P_a)\) to calculate \(\text{NMR}_{\text{ab}}\) as the gold standard, and \((P_a - P_s)\) to calculate \(\text{NMR}_{\text{ah}}\) for both normal and stenosis conditions. As discussed above, one of the most important advantages of the angiographic technique for NMR measurement is the elimination of risks associated with advancing a pressure wire across a stenotic lesion and the reduction of the procedure’s cost. Our preliminary study compared pairs of NMR calculated from \((P_a - P_s)\) and \((P_a - P_d)\) during various stages of severity of microvascular disease involving mild epicardial stenosis of up to \(\sim 50\%\) area stenosis by using both flow probe and the proposed angiographic method. In 45 pairs of measurements, paired samples \(t\)-tests showed no significant difference between \(P_a\) and \(P_d\). The SEE of NMR measurements between using \(P_a\) and \(P_d\) relative to the mean NMR measured was 10.7\% for \(\text{NMR}_{\text{ab}}\) and 10.5\% for \(\text{NMR}_{\text{ah}}\). Additionally, a Bland-Altman plot identified the mean NMR measured was 10.7\% for \(\text{NMR}_{\text{qh}}\) and 10.5\% for \(\text{NMR}_{\text{ah}}\).

*Study limitations.* In this study, several injections of microspheres were made. This may cause heterogeneous microinfects, which is not the same pathological change as normal myocardial infarction or diffused microvascular disease (2). Moreover, this experimental animal model only introduced a 50\% diameter stenosis in addition to the microcirculatory disruption. More severe epicardial stenosis, along with microvascular disease, should be studied in the future. Furthermore, other disease conditions, such as ventricular hypertrophy, diabetes mellitus, flow-limiting diffuse coronary artery disease, and previous myocardial infarction, should be considered. The impact of other disease conditions on NMR requires additional study.

In the case of severe stenosis, collateral flow might lead to an overestimation of NMR, because myocardial perfusion is a combination of both coronary and collateral flows. For our current angiographic flow measurement, a region of interest large enough to encompass the LAD vascular bed was drawn. This technique ensured that any visible potential collateral flow perfusion would be included in the angiographic NMR measurement. However, in cases of severe stenosis, coronary angiography still has only a limited sensitivity for quantifying collateral circulation capacity. Additionally, in a clinical setting, it is difficult to avoid using wires in cases of severe epicardial stenosis, because the lesion has to undergo intervention. Therefore, our angiographic method is more suitable for the patients with less severe stenoses for which intervention might not be required. In future studies, it will be necessary to measure the coronary wedge pressure to assess the collateral flow and incorporate it into the calculation of MR in the presence of a severe epicardial stenosis.

Another limitation of the angiographic method for flow measurement is motion misregistration. Respiratory motion can introduce misregistration artifacts in phase-matched subtracted images and increase measurement error in coronary flow. However, motion artifacts can be minimized with breath holding, since only a short time interval is required for blood flow measurement (3−5 s).

*Conclusion.* Compared with \(P_{CFR}\) and \(P_{PFR}\), NMR is a more reliable index to diagnose microcirculation disruption and to provide a more accurate assessment of microcirculation, especially when epicardial stenosis is present. Moreover, angiography-based NMR can potentially be a simpler and less invasive method for specific assessment of the coronary microvascular condition of patients with stable chest pain during routine coronary arteriography.

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**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

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