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Impact of the Coronary Flow Reduction at Rest on Myocardial Perfusion and Functional Indices Derived from Myocardial Contrast and Strain Echocardiography

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Abstract

**Background:** The severity of the coronary flow reduction that corresponds to myocardial perfusion and functional abnormalities remains unclear. We estimated the impact of various severities of flow-limiting coronary stenosis at rest on myocardial perfusion and functional indices from myocardial contrast echocardiography (MCE) and tissue strain imaging (TSI) and characterized the relationship between both the indices.

**Methods:** Four levels of flow-limiting stenoses (slight, mild, moderate, severe) of the left circumflex artery (LCx) were examined in 10 open-chest dogs. In the LCx area, plateau video intensity and time to plateau (TP) of the replenishment curve from MCE were calculated for perfusion analysis, and peak systolic strain ($\varepsilon_{\text{sys}}$) and postsystolic strain index (PSI) from TSI were measured for functional analysis.

**Results:** Plateau video intensity and $\varepsilon_{\text{sys}}$ tended to decrease with increased severity of stenosis, although these differences did not reach the level of statistical significance. TP and PSI were significantly increased in the context of moderate ($\geq 30$ to $< 50\%$) and severe ($\geq 50\%$) flow reduction when compared to baseline values (TP, moderate 1.69±0.20 and severe 1.77±0.25 vs. baseline 0.93±0.17, p<0.01, respectively; PSI, moderate 0.96±0.15 and severe 1.28±0.32 vs. baseline 0.59±0.18, p<0.05 and p<0.01, respectively). Further, TP and PSI were positively correlated with flow reduction ($r=0.81$ and $r=0.84$, p<0.0001, respectively), and PSI was positively correlated with TP ($r=0.72$, p<0.0001).

**Conclusions:** In contrast to conventional indices, such as plateau video intensity and $\varepsilon_{\text{sys}}$, novel indices, such as TP and PSI, were both able to detect $\geq 30\%$ coronary flow reduction at rest.
Reduction of the coronary flow results in regional myocardial ischemia and deterioration of myocardial function in the risk area. Recent developments in myocardial contrast echocardiography (MCE) and tissue strain imaging (TSI) have allowed for quantitative assessment of regional myocardial perfusion (1-4) and function (5-10), respectively. Although myocardial perfusion and functional indices derived from MCE and TSI may permit the sensitive detection of the coronary flow reduction that has not been able to be detected without the use of stress testing, the influence of the severity of the coronary flow reduction at rest on these indices has not been sufficiently examined. Moreover, the relationship between these indices also remains unclear. We, therefore, estimated the impact of various severities of flow-limiting coronary stenosis at rest on myocardial perfusion and functional indices from MCE and TSI and to characterize the relationship between both the indices.

METHODS

Animal Preparation

All animal studies were performed in accordance with guidelines for the care and use of laboratory animals at Osaka University Medical School, Osaka, Japan, and with the American Heart Association guidelines for use of animals in research. A total of ten open-chest dogs were used in this study. Dogs were anesthetized using intravenous pentobarbital sodium (35 mg/kg), intubated, and ventilated with room air using a respirator pump. Anesthesia was maintained throughout the experiment (6-8 mg/kg/h). An 18-gauge peripheral intravenous catheter was positioned in the foreleg for administration of contrast agent. A 5F catheter was placed in the thoracic aorta for monitoring of blood pressure, and a 7F catheter was placed in the femoral vein for
administration of intravenous medications and fluids. Electrocardiography was monitored continuously.

Briefly, a left lateral thoracotomy was performed, and the heart was suspended in a pericardial cradle. The proximal portion of the left circumflex coronary artery (LCx) was dissected free from surrounding tissues, and a vascular occluder was placed to induce coronary stenosis. A perivascular ultrasonic flow probe was placed at the distal site of the coronary occluder and was connected to a digital flowmeter (Transonic Systems, Ithaca, N.Y.). The position of the ultrasound transducer (PST-30BT, Toshiba, Otawara, Japan) was fixed at the midpapillary muscle short-axis view using a mechanical arm.

**TSI**

TSI was performed using the Aplio ultrasound system (Toshiba, Otawara, Japan). Tissue harmonic two-dimensional images (transmitting at 2.0 MHz and receiving at 4.0 MHz) and tissue harmonic Doppler data (transmitting at 1.55 MHz and receiving at 3.1 MHz) were captured over three consecutive cardiac cycles. The sector angle of the tissue Doppler was set at 60°, and the frame rate was set at 103 frames/s. All data were digitally stored on the system.

Myocardial radial strain was analyzed from tissue Doppler data using online software (TDI-Q, Toshiba, Otawara, Japan). In brief, myocardial displacement of an ultrasound beam direction was calculated using temporal integration of the velocity at each myocardial point on the beam. In this system, the tissue Doppler tracking technique provided by the software allows accurate measurement of the displacement. The accuracy of displacement is validated by an in vitro study (11). Strain value
between two points on a beam was determined using the strain-definition formula and
assigned at the endocardial side of the two points. The distance between two points
(derivative pitch) was set at 4 mm. After the center of contraction was manually set on
the image, Doppler angle correction was performed to evaluate regional radial strain in
the short-axis view. The reference point (i.e., zero strain) was set at the peak R wave in
electrocardiography. Assigned strain values in the myocardium were color-coded and
displayed on two-dimensional images. Timing of aortic valve closure (AVC) was
determined by phonocardiogram.

In the present dog model, the center of the LCx region was typically located at
the 3 o’clock position of the short-axis view. Despite the angle correction technique,
accurate measurement of regional radial strain was difficult at this segment because of
the excessive Doppler angle. Therefore, a pair of 4-mm circular regions of interest
(ROIs) was placed at the 4 to 5 o’clock position for the analysis of the LCx region and
at the 7 to 8 o’clock position for the analysis of the region perfused by left anterior
descending artery (LAD). Positioning of the ROI within the LCx or LAD region was
confirmed by real-time MCE performed during transient LCx occlusion. The ROI was
set to be touched on the endocardium and manually tracked, frame by frame, during
cardiac cycles. Because myocardial thickness of the dogs that used in this study was
approximately 8 mm and the strain value was assigned at the endocardial side of 4-mm
derivative pitch, the following TSI data from the 4-mm ROI that was set on the
endocardial side hardly include Doppler information beyond myocardium.

Peak systolic strain ($\varepsilon_{\text{sys}}$) in the ROI was measured as a quantitative parameter
for contractile function (Figure 1). The parameter, $\varepsilon_{\text{sys}}$, of the LCx region was corrected
by that of the nonischemic LAD region (i.e., $\varepsilon_{\text{sys}}$ in the LCx / $\varepsilon_{\text{sys}}$ in the LAD), because
myocardial contraction at baseline varied in each dog. Postsystolic thickening (or shortening) is a highly sensitive marker of myocardial ischemia (7-9). Thus, peak postsystolic strain ($\varepsilon_{\text{post}}$), defined as the peak strain coincident with postsystolic thickening after AVC, was also measured (Figure 1). The postsystolic strain index (PSI), defined as the ratio $\varepsilon_{\text{post}}/\varepsilon_{\text{sys}}$, was subsequently calculated. When there was no postsystolic thickening, PSI was calculated as $\varepsilon_{\text{post}}$ using the strain value at the regional onset of myocardial thinning caused by early mitral filling. The onset of thinning was assessed from tissue Doppler data. The values from three consecutive cardiac cycles were averaged to obtain each parameter.

**MCE**

Real-time MCE (24 frames/s) was performed using the same Aplio system. Images were acquired with the pulse subtraction mode (transmitting at 1.8 MHz and receiving at 3.6 MHz) at the same short-axis view as used for TSI. The overall gain setting and image depth were optimized at the beginning of each experiment. The dynamic range was 40 dB, and beam focus was set at the posterior wall of the left ventricle. All settings were kept constant throughout each experiment. Definity (Bristol-Myers Squibb Medical Imaging, Billerica, Mass.) was diluted 1:10 in normal saline and administered intravenously at a rate of 0.75 mL/min. After a steady state of myocardial opacification was reached, five ultrasound pulses at a mechanical index of 1.6 were transmitted to destroy the myocardial microbubbles, followed automatically by imaging with a mechanical index of 0.1. All MCE images were recorded on videotape with an S-VHS recorder for subsequent offline analysis.

Background-subtracted myocardial contrast video intensities (256 gray scales)
in end-systole were analyzed from MCE data using an offline Color Cardiology Work Station (Tomtec, Boulder, Colo.). A pair of 4-mm circular ROIs was placed at the same position as that used in the TSI analysis. Using Origin 6.0 software (Microcal, North Hampton, Mass.), contrast video intensity versus time plots were generated and fitted to an exponential function, \( y = A(1-e^{-\beta t}) \), where \( y \) is video intensity at time \( t \), \( A \) is the plateau video intensity representing myocardial blood volume, and \( \beta \) reflects mean microbubble velocity (1). The reciprocal of \( \beta \) (ie, \( 1/\beta \)), which represents the time to plateau (TP), and \( A \) values were used as quantitative parameters for myocardial perfusion (Figure 1). TP and \( A \) values on the LCx region were corrected by those on the nonischemic LAD area as well as by \( \varepsilon_{sys} \) (ie, TP or \( A \) in the LCx / TP or \( A \) in the LAD). These indices derived from MCE were analyzed by another observer who was blinded to the TSI data.

**Experimental Protocol**

Dogs with well-developed collateral vessels were excluded from study. The presence of natural collateral flow to the risk area was determined by real-time MCE during transient LCx occlusion. Based on our previous data (12), well-developed collateral perfusion was defined as an increase in video intensity in the risk area 15 seconds after high power pulses that was >15% of that in non-ischemic area.

The LCx was narrowed during monitored coronary flow to generate up to four levels of flow-limiting stenosis: slight (<15% reduction), mild (≥15 to <30%), moderate (≥30 to <50%), and severe (≥50%). TSI and MCE data were acquired at least 5 minutes after creating each stenosis to stabilize the systemic and coronary hemodynamics. After data acquisition at each level, LCx flow was measured again to confirm stenosis severity, and the stenosis was subsequently relieved. Hemodynamics and myocardial contraction...
were allowed to return to the baseline level before proceeding to the next stenosis setting. TSI was always performed prior to MCE to avoid any influence on microbubbles on tissue Doppler data. At the end of the experiment, the dog was euthanized with an overdose of pentobarbital and KCl.

**Interobserver and Intraobserver Correlations**

Ten TSI and MCE data sets were randomly selected from baseline and each stenosis setting. To determine the interobserver correlations for PSI and TP measurements, data analysis was repeated by a second observer who was blinded to the values obtained by the first observer. To assess intraobserver correlations, the analysis of the data was repeated 2 weeks later by the same observer.

**Statistical Analysis**

Data were expressed as mean±SD. The comparison of hemodynamics and indices derived from TSI and MCE between baseline and each level of stenosis was performed by ANOVA and the post hoc Dunnet’s test. The correlation of each indices derived from TSI and MCE with flow reduction and the correlation between TSI and MCE indices was determined using least squares fit regression. Values of p<0.05 were considered to represent statistical significance.

**RESULTS**

Well-developed collateral flow was observed using real-time MCE in three of ten dogs during transient LCx occlusion. These dogs were excluded from study. In one dog, MCE was incomplete because of insufficient recording. Therefore, a total of six
dogs were included in final analysis.

Heart rate and systolic/diastolic blood pressure were not significantly different when comparing values at each level of flow-limiting stenosis (Table 1). Absolute value of the LCx flow gradually decreased from 19.5±4.1 mL/min at baseline to 5.5±1.3 mL/min in severe flow reduction.

In the perfusion analysis, TP values significantly increased in the moderate and severe flow reduction in comparison to baseline (p<0.01 in moderate and severe). By contrast, $A$ values from the replenishment curve tended to decrease only in the severe flow reduction condition, although the difference was not statistically significant (Table 1). In the functional analysis, $\varepsilon_{\text{sys}}$ tended to decrease with increasing severity of coronary stenosis, but this difference did not reach the level of statistical significance. PSI significantly increased in the moderate and severe reduction conditions when compared to baseline (p<0.05 in moderate, p<0.01 in severe; Table 1).

Tissue strain images at end-systole and the strain profiles in one cardiac cycle at baseline and during severe flow reduction of the LCx are shown in Figure 2. The tissue strain image at baseline demonstrated that yellow or orange color was mainly assigned on subendocardial myocardium because myocardial end-systolic strain in the subendocardium is greater than that in the epicardial side. During the severe flow-limiting stenosis, the tissue strain image showed that end-systolic strain had decreased in the LCx area. Although strain profiles in the LCx and LAD regions were similar at baseline, the LCx strain profile during the severe flow reduction condition indicated decreased $\varepsilon_{\text{sys}}$ and marked postsystolic thickening.

Real-time MCE images and replenishment curves in the LAD and LCx regions during moderate flow reduction of the LCx are demonstrated in Figure 3. During the
moderate flow-limiting stenosis, contrast opacification in the LCx region after the high power pulses was delayed as compared to that in the LAD region. Therefore, TP in the LCx region was clearly prolonged in the analysis of replenishment curves. However, plateau video intensities (ie, $A$ values) in both the regions were almost the same in this stenosis setting.

There was a significant positive correlation between TP and flow reduction ($r=0.81$, $p<0.0001$), and a significant positive correlation between PSI and flow reduction ($r=0.84$, $p<0.0001$). However, there was no significant correlation of $A$ and $e_{sys}$ with flow reduction (Figure 4). In the relationship between myocardial perfusion and functional indices, there was a significant positive correlation between PSI and TP ($r=0.72$, $p<0.0001$); however, there was no significant correlation between the other indices (Figure 5).

The interobserver and intraobserver correlations for PSI measurement were $r=0.77$, $p<0.01$ and $r=0.78$, $p<0.01$, respectively. Those for TP measurement were $r=0.74$, $p<0.05$ and $r=0.71$, $p<0.05$, respectively.

**DISCUSSION**

MCE allows detecting non-flow-limiting stenosis using a stress test of the hyperemic response (3, 4). Without the use of any stress stimulus, however, the severity of coronary flow reduction that can be estimated by MCE is still unclear. Masugata et al. reported that there was no difference in the contrast video intensity of a long pulsing interval when comparing baseline values to those obtained during a 75% reduction in flow. By contrast, use of MCE with shorter pulsing intervals was able to adequately characterize states of severe stenosis (2). In the present study, $A$ values from the
replenishment curve did not significantly vary with the increasing levels of stenosis severity, but TP (ie, 1/ß) did increase with the increasing severity of the flow reduction. These data suggest that myocardial blood volume was maintained with severe flow-limiting stenosis but that the blood flow speed in the microcirculation was greatly reduced in response to reductions in coronary flow at rest.

TSI can also permit quantitative assessment of stenosis severity (7, 10). Jamal et al. demonstrated that PSI obtained at rest in a pig model increased with the severity of coronary stenosis, while the $\varepsilon_{\text{sys}}$ did not change unless stenosis reached the level subtotal coronary occlusion (7). Previous reports in animal and human studies have suggested that postsystolic thickening is highly sensitive marker of ischemia (7-9). This is consistent with the TSI results in the present study, which demonstrated that $\varepsilon_{\text{sys}}$ was not a good marker of coronary flow reduction. Further, the present study suggested that conventional indices of echocardiography, such as segmental wall motion and wall thickening, might not vary with a change to moderately low flow in the coronary arteries at rest.

In previous reports, the utility of MCE and TSI for characterizing flow-limiting stenosis has been investigated independently. This is the first study to investigate the relationship between quantitative perfusion indices derived from MCE and quantitative functional indices derived from TSI. The present study demonstrated that TP and PSI values both significantly increased in the context of moderate and severe flow reduction states. Further, there was a significant correlation between TP and PSI in the case of flow-limiting coronary stenosis. Of particular note, the degree of deterioration of myocardial blood flow speed correlated with the magnitude of delayed contraction after aortic valve closure.
Recent studies have shown that MCE is more sensitive than conventional wall motion analysis for the detection of ischemia (13, 14). However, the present results suggest that MCE and TSI can similarly detect greater than a 30% reduction in coronary flow when TP and PSI are used in the absence of exercise or pharmacological stress testing. Thus, the sensitivity of wall motion analysis may be improved using novel parameters, such as PSI.

**Limitations**

The present study excluded dogs with collateral circulation, because the measured flow volume by the flow meter does not adequately reflect the degree of myocardial ischemia of the area at risk in the presence of collateral vessels. Therefore, these data cannot be applied to cases in which extensive collateral vascular supply is present.

Up to four different levels of flow-limiting stenoses were assessed in each dog. Although, the animals were allowed to recover for a period of time after induction of each levels of stenosis, it is possible that the sequential challenges could lead to tolerance to myocardial ischemia or myocardial stunning. Rather than applying the various levels of coronary stenosis in a random order, levels of stenosis were sequentially induced from mild to severe to avoid any subsequent changes in the model after induction of severe flow reduction. However, we believe the given intervals were sufficient, judging from the changes in PSI during the recovery periods. Further, previous reports have demonstrated that PSI is higher in the reperfused myocardium (15, 16), suggesting that myocardial stunning did not occur in the present experiments.

In the present study, we did not test non-flow-limiting stenosis during stress
testing (ie, demand ischemia). During stress testing, myocardial ischemia is affected by myocardial oxygen consumption rather than flow reduction. Because severe ischemia may be induced by greater stress even in mild flow reduction, the demand ischemia model may complicate the impact of flow reduction on MCE and TSI indices. Thus, we used only the model of flow-limiting stenosis.

**Clinical implications**

Although coronary flow reduction due to flow-limiting stenosis is a serious clinical condition, conventional methods are not sufficient to diagnose this condition. The present data suggest that TP and PSI analysis permit sensitive detection of flow-limiting stenosis, even in the absence of stress testing. When using either modality alone, MCE is not sufficient for the assessment of posterior myocardial perfusion because of signal attenuation (17), whereas TSI is limited in estimating apical wall motion due to the angle dependency (18). The combination of both methods may compensate for these limitations and improve the sensitivity and specificity for detecting ischemia; however, further studies in a large number of patients are needed to confirm them.

**Conclusions**

MCE and TSI allow quantitative assessment of perfusion and function in the regional myocardium. In contrast to conventional indices, such as $A$ and $\varepsilon_{sys}$ values, novel indices, such as TP and PSI derived from MCE and TSI, were both able to detect $\geq30\%$ coronary flow reduction at rest. The significant correlation between TP and PSI was shown during flow-limiting coronary stenosis.
REFERENCES


Soc Echocardiogr 2001;14:874-84


FIGURE LEGENDS

Figure 1  (A) A strain profile during one cardiac cycle. Peak systolic strain ($\varepsilon_{sys}$) and peak postsystolic strain ($\varepsilon_{post}$), defined as the peak strain coincident with postsystolic thickening after aortic valve closure (AVC), were analyzed for contractile function. The postsystolic strain index (PSI), defined as the ratio $\varepsilon_{post}/\varepsilon_{sys}$, was subsequently calculated.  

(B) A replenishment curve from contrast video intensity versus time plots. The plots were fitted to an exponential function, $y=A(1-e^{-\beta t})$. The reciprocal of $\beta$, which represents the time to plateau (TP), and $A$ values were analyzed for myocardial perfusion.

Figure 2  Tissue strain images in end-systole, and the strain profiles in a cardiac cycle at baseline (A, B) and during severe flow reduction in the left circumflex artery (LCx) (C, D). During the flow reduction, the orange color within the region of interest placed in the LCx region became darker compared to that in the left anterior descending artery (LAD) region, indicating that the strain value in the LCx region was decreased at end-systole (C). Strain profile in the LCx region expressed as decreased peak systolic strain and marked postsystolic thickening during ischemia (D). A vertical yellow line indicates aortic valve closure.

Figure 3  A sequential change of myocardial contrast opacification in end-systole during moderate flow reduction of the left circumflex artery (LCx) (A to F) and the replenishment curves in the left anterior descending artery (LAD) and LCx regions. Microbubbles within myocardium were completely destroyed just after the high power pulses (A). Relatively rapid contrast opacification was observed in the whole of myocardium except the LCx region (B, C), and delayed opacification was shown in the LCx region (D to F). TP in the LCx region was clearly prolonged in the analysis of replenishment curves. However, plateau video intensities (ie, $A$ values) in both the
regions were almost the same in this stenosis setting.

**Figure 4**  Relationship of time to plateau (TP), postsystolic strain index (PSI), $A$ value, and peak systolic strain ($\varepsilon_{\text{sys}}$) with flow reduction in the left circumflex artery.

Significant positive correlations were shown in TP and PSI but not in $A$ value and $\varepsilon_{\text{sys}}$.

**Figure 5**  Relationship between myocardial perfusion indices, such as time to plateau (TP) and $A$ value, and functional indices, such as postsystolic strain index (PSI) and peak systolic strain ($\varepsilon_{\text{sys}}$), during flow-limiting stenosis. A significant positive correlation between PSI and TP was demonstrated but not between the other indices.