Fluorescent Cardiac Imaging

A Novel Intraoperative Method for Quantitative Assessment of Myocardial Perfusion During Graded Coronary Artery Stenosis

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Background—The purpose of the present study was to examine whether the effect of coronary stenoses of variable severity on myocardial perfusion can be quantitatively assessed in vivo by analysis of fluorescent cardiac imaging (FCI) compared with the gold standard, the fluorescent microsphere method. FCI is a novel technology to visualize coronary vessels and myocardial perfusion intraoperatively using the indocyanine green dye with an infrared-sensitive imaging device.

Methods and Results—Graded stenoses and total vessel occlusion of the left anterior descending coronary artery were created in 11 open-chest pigs. Stenoses were graded to reduce resting left anterior descending coronary artery flow by 25%, 50%, 75%, and 100% of baseline flow measured by transit-time flowmeter. FCI images were analyzed with a digital image processing system. The impairment of myocardial perfusion was quantified by background-subtracted peak fluorescence intensity and slope of fluorescence intensity obtained with FCI and compared with myocardial blood flow assessed by fluorescent microsphere. All stenoses resulted in an impairment of myocardial perfusion visualized by FCI. Occlusion of the left anterior descending coronary artery resulted in a total perfusion defect (no fluorescence intensity) of the corresponding anterior myocardial wall. During graded stenosis and total vessel occlusion, normalized background-subtracted peak fluorescence intensity and slope of fluorescence intensity decreased significantly \((P<0.0001)\). Both background-subtracted peak fluorescence intensity \((r=0.92, P<0.0001)\) and slope of fluorescence intensity \((r=0.93, P<0.0001)\) analyzed by FCI demonstrated good linear correlation with fluorescent microsphere–derived myocardial blood flow.

Conclusions—The impairment of myocardial perfusion in response to increased coronary stenosis severity and total vessel occlusion can be quantitatively assessed by FCI and correlates well with results obtained by fluorescent microsphere. (Circulation. 2007;116:1007-1014.)

Key Words: angiography ■ bypass ■ imaging ■ microspheres ■ perfusion

About 67,500 patients in Germany and \(\approx400,000\) patients in the United States undergo coronary bypass surgery each year.\(^1\) In recent years, alternative coronary artery bypass grafting (CABG) techniques such as arterial grafting, off-pump coronary artery bypass procedures, minimally invasive direct coronary artery bypass, evolving anastomotic devices, and endoscopic CABG have increased in popularity. Most graft failures occur early and are most likely caused by technical problems.\(^2\) Thus, intraoperative or immediate postoperative quality control of anastomoses and grafts is mandatory to identify early technical problems and to obtain good short- and long-term results.

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At present, there are several technologies to assess intraoperative bypass function, including intraoperative coronary angiography, thermal coronary angiography, ultrasound transit-time flow measurement (TTFM), and high-frequency epicardial echocardiography. However, all these techniques have limitations such as invasivity, misinterpretation, or poor resolution, and the clinical impact of most of these methods remains an unsolved issue.\(^2,3\)

Recently, we described the value of a novel, indocyanine green (ICG)–based imaging system (LLS GmbH, Ulm, Germany) for intraoperative visualization of blood flow in
coronary vessels and bypass grafts. Other groups also have shown the feasibility of this technique to monitor the quality of the bypass graft in CABG using a similar imaging device (SPY, Novadaq Technologies, Toronto, Canada). However, direct evaluation of anastomoses seems to be less accurate owing to the presence of adjacent tissue and the limited depth of penetration of the laser beam. Thus, indirect parameters to detect anastomotic stenoses were evaluated in the present study.

Our group has previously shown that this technique also allows the visualization of myocardial perfusion, providing further information with respect to the blood supply to the corresponding myocardium. Visualized by fluorescent cardiac imaging (FCI), coronary stenoses resulted in an impairment of myocardial perfusion, and vessel occlusion resulted in a total perfusion defect of the corresponding myocardial region. Although high-quality images of myocardial perfusion were obtained by FCI, quantitative assessment of myocardial perfusion deficits was not yet possible.

The aims of the present study were to quantitatively assess the effect of different coronary stenoses on myocardial perfusion by analysis of FCI and to compare the results with myocardial blood flow (MBF) assessed by the gold standard, fluorescent microspheres (FMs).

Methods

Animal Experiments

The experiments were performed in 11 open-chest pigs of either sex weighing between 28 and 40 kg at the Institute for Surgical Research, Ludwig-Maximilians University (Munich, Germany). The study was approved by the governmental animal care committee and the institutional review board for the care of animal subjects. All animal studies were performed in accordance with the “Position of the American Heart Association on Research Animal Use.”

General anesthesia was by dormicum (0.1 mg/kg), fentanyl (6 μg · kg-1 · h-1), and isoflurane (1.0% to 1.5%). Arterial blood gas analyses were performed regularly to ensure adequate oxygenation. A 4F arterial catheter with an embedded thermistor was inserted into the right femoral artery for continuous hemodynamic monitoring of stroke volume, cardiac output, cardiac index, systemic vascular resistance, and arterial blood pressure by the determination of thermodilution. These parameters were documented by the PiCCO device (Pulsion Medical Systems, Munich, Germany) on the basis of arterial pulse contour analysis. A double-lumen central venous catheter was inserted into the right jugular vein for delivery of infusions, heparin, and ICG. For application of the FM, a catheter was placed in the left atrium. A pigtail catheter was inserted into the abdominal aorta via the right carotid artery for withdrawal of reference blood samples with a known constant withdrawal rate to calculate the rate of flow per tissue sample.

The heart was exposed through a median sternotomy. Deep pericardial traction sutures were placed on the left side for exposure of the left anterior descending coronary artery (LAD). The proximal segment of the LAD was dissected free from surrounding tissue, and a 2-mm TTFM probe (CardioMed Flowmeter, Medi-Stim AS, Oslo, Norway) was placed around the vessel for flow measurements. Graded stenoses were produced by a custom-designed screw occluder. The degree of the LAD stenoses was determined by proportional reduction with respect to the baseline flow. Coronary artery flow was measured with the TTFM at baseline, during graded stenosis, and after total vessel occlusion.

Myocardial Perfusion Measurement With FCI

Myocardial perfusion was assessed with the FCI system (LLS GmbH). The technology is based on the fluorescence of ICG, a nontoxic, water-soluble fluorescent dye that has been used extensively for >40 years for examination of liver function, microcirculation, and ophthalmic angiography. The dye immediately binds to plasma proteins and remains intravascular after systemic injection. Because it is rapidly eliminated exclusively by the liver (half-life, 2.4 minutes), repeated injections of ICG are possible. Adverse reactions related to its clinical use are rare, and side effects other than iodine allergy have not been reported. In plasma, it displays an absorption maximum at 805 nm and an emission maximum at 830 nm.

The ICG fluorescent dye was administered intravenously through a central venous line to ensure adequate mixing of the ICG. The dose of ICG was adapted to body weight (0.03 mg/kg body weight), indicating the exact amount of ICG injected. The heart was illuminated with near-infrared light at a wavelength of 785 nm provided by infrared laser diodes with a total output of 80 mW in a field of view of 10 cm in diameter. Because of the power density of the emitted laser energy, there is no tissue warming. Eye protection is not required in the operating room because the laser light energy is dispersed and the light remitted from the tissue surface (30% of the incident light) has no hazardous potential (1 mW/cm2). The fluorescence emission of the excited dye was detected by an infrared-sensitive charged-couple device camera system equipped with a band-pass filter for the selective transmission of light at the central wavelength of 830 nm. The dynamic range of the camera was 54 dB. The signals of the camera were digitized with a frame grabber card that provides a resolution of 8 bits. Images were acquired at a rate of 25 frames per second and were recorded in real time. All measurements were performed under standardized conditions, with identical optical and hemodynamic parameters. The charged-couple device imaging system was positioned on the exposed surface of the heart at a distance of ~25 cm. Camera distance was measured and calibrated after each measurement. The FCI images were displayed in real time on a computer monitor and analyzed using a digital image processing system with a temporal resolution of 20 ms and spatial resolution of ~0.2 nm at a penetration depth of 4 nm (LLS GmbH).

MBF Measurements With FMs

FMs 15 μm in diameter (Molecular Probes, Eugene, Ore) were used to measure MBF after each intervention. Microspheres labeled with different fluorescent colors were randomly selected for application. Excitation and emission wavelengths for each of the fluorescence microspheres were as follows: blue, 356/424 nm; blue-green, 427/468 nm; yellow-green, 495/505 nm; orange, 534/554 nm; red, 579/638 nm; and scarlet, 651/680 nm. They did not interfere with the excitation and emission wavelength of ICG.

Each vial of FMs was placed in an ultrasonic water bath for 5 to 10 minutes to disperse the microspheres and vortexed twice for 3 minutes to ensure proper mixing before injection. Approximately 5×105 FMs were suspended in physiological saline solution to a volume of 10 mL and injected constantly over 60 seconds into the left atrium at each intervention. Reference blood samples were withdrawn in anticoagulated (5 mL of 3.13% sodium citrate) syringes from the abdominal aorta through a pigtail catheter (7F, Cordis, Miami, Fla) with a constant-rate withdrawal pump at 3.18 mL/min over 3 minutes for calculation of MBF per tissue sample (model 640A, Harvard Apparatus, South Natick, Mass). To assess myocardial perfusion, FMs have to be injected into the left atrium. Injection of FMs was started when the withdrawn blood reached the suction syringe. At the end of the experiments, the pigs were killed, and the hearts were excised and fixed in 10% formaldehyde for 6 to 8 days. The anterior wall of the left ventricle was isolated and dissected into samples of equal size. The myocardial slices correspond to a reticle projected onto the FCI computer images.

The left anterior wall was dissected into 20 wedge-shaped transmural tissue pieces with a mean weight of 1.5±0.8 g. The slices were divided into epicardial and endocardial segments, and the specimens were processed for determination of MBF by spectrophotometry according to the standard method described by Glenny et al.
Processing of the tissue samples was completely automated by a modified Zymate Robotic System (Zymark, Idstein, Germany). The robotic system was used to recover the FM of the tissue samples so that the fluorescence intensities could be measured with a luminescence spectrophotometer and an automated sampling unit (Perkin Elmer, Überlingen, Germany) to calculate organ blood flow.15,16

Experimental Protocol
Baseline hemodynamic measurements were recorded and the LAD flow was measured with the TTFM when hemodynamic stability had been achieved. ICG and FMs were injected to determine baseline myocardial perfusion by FCI and MBF of the LAD territory. Next, the LAD diameter was reduced progressively to produce 3 levels of graded stenoses with a flow reduction of 25%, 50%, and 75% of the baseline value. After each intervention, the stenosis was removed so that flow returned to baseline and the hemodynamic parameters were stabilized. Finally, the LAD was completely occluded to document the total ischemic myocardial area. ICG and FMs were injected at each intervention for FCI and MBF measurements. Time interval between stenosis induction and FCI varied between 5 and 15 minutes because of hemodynamic and coronary flow stabilization. The time elapsed between the injections was ~30 minutes and varied between 25 and 40 minutes. Stable hemodynamic parameters were essential for comparisons of TTFM, FM, and FCI data among the 5 flow states. Mean total experimental time was 4.6 hours.

Arterial blood gas and hemodynamic variables were monitored continuously and were kept within normal limits.

Image and Data Analysis
At the very low concentrations of ICG used in our experiments, the fluorescence intensity is proportional to the time-dependent concentration of ICG in the blood and the blood volume in the tissue because quenching effects are negligible.17 Thus, the amount of ICG in the blood is represented by the pixel intensity of the image. To obtain a quantitative measure for the fluorescence intensity, we calculated both the mean value and the SD of the measured pixel intensities in the region of interest on the myocardial wall for each image in a sequence of 60 seconds after the injection of ICG. This region of interest was 30×30 mm on the myocardial surface, depending on the distance from the camera. The concentration of the dye in the blood during the first passage through the myocardium is normally distributed as represented by a Gaussian curve. Before the second passage through the myocardium, the concentration of the dye is homogeneus in the whole-blood volume with an exponential decay in time that is typical for hepatic excretion. Thus, the temporal course of the fluorescence intensity can be fitted by a combination of a Gaussian curve and a curve with an exponential decay. Adapting the free parameters of this model with a least-squares fit to the measured intensity values allows elimination of the artifacts caused by the beating heart and respiration cycle and facilitates calculation of the slope of the intensity and other relevant parameters. To measure myocardial perfusion, we calculated 2 different parameters derived from the time-dependent fluorescence signal.

Background-Subtracted Peak Fluorescence Intensity
To calculate background-subtracted peak fluorescence intensity (BSFI) from the time-dependent fluorescence intensity, the initial intensity value before the injection of ICG was subtracted from the peak fluorescence intensity during the first passage of the dye through the myocardium.

Slope of Fluorescence Intensity
This parameter is represented by the maximal slope during the increase of the time-dependent fluorescence intensity induced by the first wave of the dye, which passes the capillaries of the myocardium. Each FCI sequence was recorded online for 60 seconds with real-time digitizing. BSFI and slope of fluorescence intensity (SFI) data were analyzed offline directly after the images were recorded with a customized software package (LLS GmbH). Data analysis for each FCI sequence took <2 minutes.

To examine the feasibility of the FCI technique for quantitative assessment of myocardial perfusion resulting from coronary stenoses of variable severity, BSFI and SFI data were correlated to FMs. FCI images were analyzed with an image processing system (LLS GmbH). The reticulated FCI computer images were then matched with the corresponding myocardial slices of the anterior wall of the left ventricle. Because we knew where the myocardial slice had been located on the myocardium, we calculated our FCI intensity data from the corresponding area (region of interest) in our fluorescence images.

In each image, a constant value of background intensity calculated from the mean residual intensity in the region of interest before ICG injection was subtracted from the peak intensity in the same region after each injection of ICG.

Statistical Analysis
Data from all animals were expressed as mean±SD. Comparisons of hemodynamics, TTFM, FM, and FCI data among the 5 flow states were performed by ANOVA. The dependence of hemodynamics, TTFM, FM, and FCI data on the percent flow was studied by fitting random slope models with an intercept of 0 to the data, allowing for heteroscedasticity in the individual measurements. The estimated mean slopes of these random-effects models are reported, accompanied by 95% CIs and probability values of tests of 0 slope. The chosen models take into effect that repeated measurements were performed within the same animal. Nonzero slopes prove that the association is nonrandom. Correlations between TTFM, FM, and FCI data were tested with linear regression analysis. In addition, the limits of agreement between the different methods were calculated as described by Bland and Altman. A value of P<0.05 was considered statistically significant. Statistical analysis was performed with the SPSS statistical software package 13.0 (SPSS Inc, Chicago, Ill).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
High-quality FCI images of the myocardial perfusion and coronary arteries were obtained in all animals. Systemic hemodynamic parameters were documented continuously. The hemodynamic parameters obtained during all protocols are summarized in the Table. No significant changes with respect to any of the hemodynamic parameters occurred during the experiments.

Severity of Coronary Stenoses
Figure 1 shows individual LAD blood flow assessed by TTFM for baseline and 4 different degrees of stenosis. Mean decrease per 25% flow reduction was 7.0 mL/min (95% CI, 5.7 to 8.3; P<0.0001). As expected, total occlusion resulted in a 100% flow reduction.

Assessment of MBF by FMs
Figure 2 shows individual MBF as assessed by FMs at baseline and 4 different degrees of stenosis (25%, 50%, 75%, and 100% flow reduction). Mean decrease per 25% flow reduction was 0.240 mL·min⁻¹·g⁻¹ (95% CI, 0.195 to 0.284; P<0.0001).

Assessment of Myocardial Perfusion by FCI
The impairment of myocardial perfusion was quantified by BSFI and SFI obtained with FCI. Figure 3 shows the background-subtracted FCI images of the myocardial perfu-
Intraoperative Hemodynamic Parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>25% FR</th>
<th>50% FR</th>
<th>75% FR</th>
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<tr>
<td>CO, L/min</td>
<td>3.2±0.8</td>
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<td>CI, L·min⁻¹·m⁻²</td>
<td>2.9±0.5</td>
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<td>HR, bpm</td>
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<td>SV, mL</td>
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<td>49.0±5.7</td>
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<td>BP syst, mm Hg</td>
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<td>67.6±8.3</td>
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<td>62.5±10.1</td>
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<td>BP dia, mm Hg</td>
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<td>BP mean, mm Hg</td>
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<td>SVR, dynes·s·cm⁻⁵</td>
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<td>939±194</td>
<td>889±197</td>
<td>918±220</td>
<td>910±232</td>
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</tbody>
</table>

FR indicates flow reduction; CO, cardiac output; CI, cardiac index; HR, heart rate; SV, stroke volume; BP syst, systolic blood pressure; BP dia, diastolic blood pressure; BP mean, mean blood pressure; and SVR, systemic vascular resistance. Values are mean±SD.

The baseline, homogeneous perfusion of the corresponding myocardium was observed. Increasing the severity of stenoses resulted in a gradual impairment of the myocardial perfusion, as measured by FCI. Occlusion of the LAD results in a total perfusion defect with no fluorescence intensity in the corresponding anterior myocardial wall, whereas myocardial perfusion of the diagonal branch and circumflex artery was preserved, showing a normal, homogeneous perfusion (Figure 3, bottom right). Thus, BSFI decreased significantly during the different flow values in all 11 animals (P<0.0001). Figure 5 (left) shows individual BSFI courses of all 11 animals obtained with FCI are depicted. BSFI decreased significantly from baseline to the different flow values in all animals (P=0.0001). The mean decrease per 25% flow reduction was 21.7 (95% CI, 17.9 to 25.5; P<0.0001).

Slope of Fluorescence Intensity

Figure 5 (right) shows individual SFI courses of all 11 animals obtained with FCI. Mean values of SFI decreased significantly from baseline to different flow values in all 11 animals (P<0.0001) by a rate of 5.4 (95% CI, 4.3 to 6.5) per 25% flow reduction (P<0.0001).

Correlation Between BSFI, SFI, and MBF

The results of linear regression comparison normalized BSFI and normalized MBF derived from a total of 55 flow states are shown in Figure 6 (left). There was good linear correlation between FM-derived MBF and BSFI during baseline and 4 different flow values in 1 representative experiment are summarized in Figure 4. During an increase in the severity of stenoses, BSFI decreased. At 100% flow reduction, no increase in fluorescence intensity analyzed by SFI could be detected at all.

Background-Subtracted Fluorescence Intensity

At baseline, the mean value of BSFI was 87±22; during 25% flow reduction, 63±16; during 50% flow reduction, 50±17; and at 75% flow reduction, 42±13; at 100% flow reduction, there was no increase in BSFI. The absolute values of the background intensity for each injection were 45.7±3.8 at baseline, 68.6±6.3 during 25% flow reduction, 83.5±6.7 during 50% flow reduction, 87.0±5.8 at 75% flow reduction, and 96.3±8.5 at 100% flow reduction. In Figure 6 (right), individual BSFI courses of all 11 animals obtained with FCI are depicted. BSFI decreased significantly from baseline to the different flow values in all animals (P<0.0001). The mean decrease per 25% flow reduction was 21.7 (95% CI, 17.9 to 25.5; P<0.0001).

Correlation Between BSFI, SFI, and MBF

The results of linear regression analysis comparing normalized SFI at baseline and during different flow values in all 11 animals (P=0.0001) correlated significantly with TTFM values. The limits of agreement between the different methods using Bland-Altman analyses are shown in Figure 7. There was good agreement between the differences of nor-

![Figure 1](image1.png)

Figure 1. Coronary blood flow of the LAD measured by TTFM at baseline and 4 graded coronary stenoses (25%, 50%, 75%, and 100% flow reduction) in 11 animals.

![Figure 2](image2.png)

Figure 2. MBF measured by FMs at baseline and 4 graded coronary stenoses (25%, 50%, 75%, and 100% flow reduction) in 11 animals.
malized BSFI and MBF and of normalized SFI and MBF from predicted values plotted against normalized MBF values. The uniform distribution of scatter above and below 0 indicates that there are no systematic differences between the 2 methods.

**Discussion**

Recently, we described a novel technology for intraoperative visualization of coronary vessels and bypass grafts using the dye ICG with an infrared-sensitive imaging device (LLS GmbH). ICG provides information in a manner similar to coronary angiography, with which the surgeon is familiar. In this new technology, radiopaque contrast media and ionizing radiation are replaced by laser light and a fluorescent dye. The fluorescence emission of the excited dye is detected by an infrared-sensitive charged-couple device camera system, displayed in real time on a computer monitor, and analyzed with digital image processing technology. The physical penetration depth of the light in myocardial tissue is ≈4 mm, where an attenuated fluorescent signal can be detected. The fluorescence intensity signal decreases exponentially with depth. Thus, only a low-fluorescence signal can be detected from the endocardial layer with ICG technology. Further studies have to demonstrate the accuracy of FCI in the assessment of endocardial ischemia.

ICG is a nontoxic dye that has been used widely for >40 years for ophthalmic angiography, measurement of liver function, and determination of cardiac output. Severe side effects are very uncommon (<1 case in 1000). In recent clinical studies with low-dose application of ICG (0.03 to 0.06 mg/kg body weight) for intraoperative quality assessment in CABG, no adverse reaction was observed. Because of the short half-life of ICG, reinjection at short intervals is possible, which enables an immediate intraoperative result. ICG binds immediately to plasma proteins and thus remains mainly intravascular after systemic intravenous injection. However, a remaining residual background fluorescence could be detected after each ICG injection. Therefore, the quantification of myocardial perfusion in the present study was based on BSFI. Furthermore, with SFI, the maximal slope during the increase of the time-dependent fluorescence intensity was measured. Thus, the influence of the background fluorescence can be neglected using the described methods for data analyzes (BSFI and SFI), which are both independent of background fluorescence.

In our previous study, we were able to show that FCI is a highly sensitive and reproducible method and an excellent technique for intraoperative quality control in CABG. Other groups also have shown the feasibility of this simple, safe, and reproducible technique to assess graft patency in the operating room using a similar ICG-based imaging device (SPY, Novadaq Technologies). Reuthebuch et al showed that the quality of coronary anastomoses and grafts could be assessed in off-pump revascularization. In this
clinical study, 107 grafts were analyzed, with ICG showing rapid filling and emptying in 103 grafts and graft failure caused by anastomotic restrictions and graft dissection in 4 grafts. Taggart et al. used this technique in 84 patients to assess 213 conduits. Fluorescence imaging confirmed graft patency in all but 4 conduits requiring graft revision. In a clinical study by Desai et al., ICG angiograms led to graft revisions for technical problems in 4.2% of patients that would have otherwise gone unrecognized. Balacumraswami et al. demonstrated that this fluorescence imaging device could detect intraoperative graft patency in off-pump and on-pump CABG. In this prospective study with 200 patients, 8 occluded bypass grafts were detected that required revision. However, direct evaluation of anastomoses seems to be less accurate because of the presence of adjacent tissue and the limited depth of penetration of the laser beam. Taggart et al. were able to clearly visualize the anastomoses in only three quarters of cases. In the study by Taggart et al., it was usually not possible to demonstrate precise anatomic detail. In fact, complete bypass occlusion can easily be visualized, but bypass stenoses with anastomotic narrowing are difficult to detect. Because of adjacent tissue, direct evaluation of anastomoses is less accurate, and skeletonized conduits permit better visualization than pedicle grafts. Furthermore, FCI provides images in only 1 plane in contrast to coronary angiography. Thus, stenoses situated parallel to the surface plane may not be visualized directly. Therefore, the FCI system could be enhanced considerably for evaluation of anastomotic quality by additional assessment of myocardial perfusion in the corresponding area. In our previous studies, we found that coronary stenoses resulted in an impairment of myocardial perfusion and that total vessel occlusion showed a complete perfusion defect of the corresponding myocardial area. In addition, reperfusion of the myocardial area became visible immediately after the release of the occlusion. Thus, this technique enabled us to detect and clearly demarcate the ischemic area.

Although high-quality images of coronary vessels and bypass grafts were obtained with FCI in different studies, this technique allowed only qualitative visualization of coronary vessels or myocardial perfusion. Studies concerning the usefulness of FCI for quantitative assessment of impaired myocardial perfusion during graft stenoses are not yet available.

The present study for the first time provides results of quantitative assessment of myocardial perfusion by FCI using ICG. The data were validated and compared with results obtained by FMs as the gold-standard technique for measurement of MBF.

Our results indicate that over a wide range of increasing severity of stenoses, impairment of the myocardial perfusion can be quantitatively assessed by FCI. Moreover, the degree of reduction in myocardial perfusion correlated well with the severity of coronary artery stenoses and correlated significantly with the degree of reduction of MBF. In the present study, myocardial perfusion could be assessed equally well by BSFI and SFI. At baseline measurement, a normal perfusion of the corresponding myocardium was observed and was verified by a rapid increase in the fluorescence intensity, resulting in high SFI and BSFI values. With increasing severity of stenoses, both BSFI and SFI values decreased significantly in each animal and therefore reflected the severity of the LAD stenoses in the corresponding myocardial area. Total occlusion of the LAD led to a visible

![Figure 5. BSFI (left) and SFI (right) obtained with FCI at baseline and 4 graded coronary stenoses (25%, 50%, 75%, and 100% flow reduction) in 11 animals.](image-url)
perfusion defect of the corresponding anterior myocardial wall, with no increase in fluorescence intensity documented by SFI and BSFI values of 0.

To compare interindividual values, BSFI and SFI were normalized. With the present study, we can demonstrate that the impairment of myocardial perfusion resulting from graded coronary stenoses measured by normalized BSFI and SFI showed good correlation with results obtained by normalized FM-derived MBF. Thus, normalized BSFI and SFI correctly depicted the progressive reduction of regional MBF produced by graded flow-limiting coronary stenoses and could differentiate between low- and high-grade flow-limiting stenoses in all cases. However, the slope of the line in linear regression analysis was <1. That means that the BSFI and SFI values compared with MBF values are “too high” for high-grade stenoses and “too low” for low-grade stenoses. This might be caused by an underestimation of the averaged MBF values for high-grade stenoses resulting from the strong decrease in endocardial perfusion, whereas the fluorescence intensity in the FCI technique is dominated by the perfusion in the epicardial layer. Furthermore, inconsistencies in the measurement of FM could result in deviations: loss of microspheres during digestion, filtering of the samples or sample handling, and spillover from each fluorescent color band to the adjacent color bands. BSFI and SFI were equally able to identify stenoses of different severity with no significant differences and correlated significantly with normalized TTFM measurements. The Bland-Altman analyses showed that in general there is good agreement between the impairment of myocardial perfusion resulting from flow-limiting coronary stenoses measured by normalized BSFI and MBF (right) from predicted value plotted vs normalized MBF values. Solid line shows the mean difference; dotted lines, limits of agreement (±2 SD). a.u. indicates arbitrary units.

**Study Limitations**

In the present study, standard curves for normally perfused myocardium and impairment of myocardial perfusion during graded coronary artery stenosis were demonstrated and validated in pigs under perfectly controlled experimental conditions. Assessment of myocardial perfusion using FCI is dependent on hemodynamic and optical parameters such as fluorescence homogeneity, position and movement of the heart, and overlying fat tissue. In this experimental study, however, these parameters did not affect the results.

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**Disclosures**

None.

**References**


**CLINICAL PERSPECTIVE**

Intraoperative quality control after coronary bypass surgery is mandatory to identify early technical problems, to avoid serious complications resulting from graft failure, and to obtain good short- and long-term results. Fluorescent cardiac imaging (FCI) is a novel, highly sensitive technology using the indocyanine green dye with an infrared-sensitive imaging device for intraoperative visualization of coronary vessels and bypass grafts similar to x-ray coronary angiography. However, direct evaluation of anastomoses is limited by images in only 1 plane, the presence of adjacent tissue, and the limited penetration depth of the laser beam. Thus, indirect parameters to detect anastomotic stenoses were evaluated in the present study. Our group has previously shown that this technique allows the visualization of myocardial perfusion, providing further information with respect to the blood supply to the corresponding myocardium. In the present study, quantitative assessment of myocardial perfusion during graded coronary artery stenosis was studied in 11 open-chest pigs by analyzing FCI compared with the gold standard, the fluorescent microsphere technique. Our results indicate that over a wide range of increasing severity of stenoses, impairment of the myocardial perfusion can be assessed quantitatively by FCI. The findings reveal an excellent correlation between FCI-derived myocardial perfusion and fluorescent microsphere–measured perfusion as the gold standard. These data establish the potential of FCI measurement of myocardial perfusion as a valuable diagnostic tool in the intraoperative assessment of patients with coronary artery disease. The present study lays the groundwork for clinical intraoperative studies of perfusion imaging in conjunction with fluorescent angiography.
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