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Myocardial Perfusion Reserve in Cardiovascular Magnetic Resonance: Correlation to Coronary Microvascular Dysfunction

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ABSTRACT

The present study examined the association of myocardial perfusion reserve index (MPRI) in cardiovascular magnetic resonance (CMR) with coronary microvascular dysfunction (CMD) and serum levels of markers of inflammation or endothelial activation. Twelve patients with typical angina pectoris without coronary artery disease were enrolled in this study, and CMR perfusion was analyzed using a steady-state-free-precession sequence with 3 short axis slices per heartbeat during first pass of 0.025 mmol Gadolinium-DTPA/kg body weight. The upslope of myocardial signal intensity curves was used to calculate MPRI. CMD was assessed by intracoronary Doppler flow measurement and biplane angiography. Both MPRI and CMD were assessed during endothelium-independent stimulation with intravenous adenosine and during endothelium-dependent stimulation with intracoronary infusion of acetylcholine. Serum values of soluble CD40 ligand (sCD40L), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), soluble intercellular adhesion molecule-1 (sICAM-1), and C-reactive protein (CRP) were measured.

Impaired MPRI correlated significantly with a decrease in coronary blood flow reserve after both endothelium-dependent ($p = 0.033$) and endothelium-independent ($p = 0.022$) stimulation. Serum levels above the median of all normal ranged biomarkers sCD40L, TNF-α, IL-6, sICAM-1 and CRP were associated with an impaired MPRI for stimulation with adenosine as well as acetylcholine. In multivariable analyses, sCD40L ($p < 0.001$) and TNF-α ($p = 0.011$) were significantly associated with a decrease in MPRI on adenosine, as were TNF-α ($p = 0.016$) and sICAM-1 ($p = 0.022$) for a decrease in MPRI on acetylcholine. MPRI on adenosine significantly correlated with MPRI on acetylcholine ($p < 0.001$).

Therefore, the present study demonstrates safety and feasibility of an intracoronary infusion of acetylcholine during CMR perfusion analysis, thus allowing direct assessment of endothelial dependent vasomotor function at the myocardial level by CMR. Furthermore, we show that an impaired myocardial perfusion reserved in CMR is associated with established biomarkers of early atherosclerosis and significantly correlated with CMD. CMR combined with adenosine could be proposed as a non-invasive tool to evaluate CMD.

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Patients with typical chest pain but no angiographically proven coronary artery disease exhibit markers of early atherosclerosis such as coronary microvascular dysfunction and subclinical inflammation. Elevated levels of inflammatory biomarkers and endothelial adhesion molecules are such markers of early atherosclerosis when compared to healthy subjects (1). Moreover, such markers of early atherosclerosis have been associated with an increased cardiovascular risk. As such, the severity of coronary microvascular dysfunction has been linked to the occurrence of adverse cardiovascular (2–4) and cerebrovascular events (5). Increased concentrations of C-reactive protein are associated with impaired coronary microvascular function (6, 7). In addition, elevation of inflammatory markers

or endothelial adhesion molecules is associated with an increased rate of adverse cardiovascular events during follow-up (8–16). Recently, Panting et al. (17) have shown that syndrome X patients (typical history of exertional angina, abnormal exercise electrocardiogram, normal coronary angiography), exhibited subendocardial hypoperfusion as assessed by CMR. So far, myocardial perfusion determined by CMR has not been linked to biomarkers of early atherosclerosis or coronary microvascular dysfunction.

We hypothesized an association between established parameters of early atherosclerosis with myocardial perfusion reserve in CMR for endothelium-dependent and endothelium-independent stimulation in patients with typical angina pectoris without coronary artery disease. In addition, to allow measurement of myocardial perfusion in CMR during endothelium-dependent stimulation, we evaluated safety and feasibility of intracoronary acetylcholine administration during CMR perfusion analysis.

METHODS

Coronary microvascular function

Patients with sinus rhythm, typical angina pectoris and angiographic exclusion of coronary artery disease (CAD) were included in this study. Exclusion of CAD was based on coronary angiography showing smooth vessel contours without lumen irregularities. We measured coronary microvascular function as previously described (4). Treatment with any cardiovascular medication was discontinued for at least 24 hours before measurements. Cardiac catheterisation was performed from the femoral approach using a 6-French sheath 60 cm in length with a 0.038-inch inner lumen (Cook, Bjaerverskov, Denmark). The tip of the sheath was placed at the distal end of the aortic arch. Heparin was given to achieve an activated clotting time of >280 sec. A 6-French guiding catheter (Cordis, Miami, FL, USA) was placed in the left coronary ostium. A 3.0-French infusion catheter (MicroFerret, Cook, Bjaerverskov, Denmark) was placed in the proximal part of the left anterior descending coronary artery. The tip of a 0.014-inch Doppler guide wire (Jometrics FloWire, Volcano, Belgium) was positioned 1 cm distal to the end of the infusion catheter. We repetitively measured blood flow velocity at: rest prior to any pharmacological stress; after three-minutes intravenous infusion of adenosine via an antecubital vein in a dosage of 140 μg/min/kg body weight; after an interval of five minutes to allow blood flow to return to baseline; after every three-minutes infusion of acetylcholine in increasing doses of 0.036, 0.36, 3.6 and 18 μg/mL. Acetylcholine was administered through the intracoronary infusion catheter with an infusion pump (Braun, Melsungen, Germany) set to a flow rate of 2 mL per minute. After every measurement of coronary blood flow velocity, coronary angiograms were performed at identical biplane projections, constant table level, constant focus-to-film distance and the same inspiration level of the patient. Coronary blood flow was calculated (18) using the mean of three measurements of averaged peak velocity and the mean of three biplane measurements of cross-sectional area derived from quantitative coronary analysis of a non-foreshortened 15 mm segment (Philips Integris, Philips Medical Systems, Best, The Netherlands). Coronary blood flow reserve for adenosine was calculated as the ratio of coronary blood flow after administration of adenosine to coronary blood flow at rest (19). Similarly, coronary blood flow reserve for maximum applied dose of acetylcholine was calculated. The study protocol was approved by the local ethics committee, and all patients gave written informed consent. The study complies with the Declaration of Helsinki.

Cardiovascular Magnetic resonance

Due to its high spatial resolution first-pass CMR is the best imaging technique to detect areas with reduced myocardial blood flow (17, 20). Therefore, to compare blood flow reserves with myocardial perfusion reserves, we performed CMR immediately after measurement of coronary microvascular function. We used a 1.5-Tesla whole body scanner (Intera CV, Philips Medical Systems) with Master gradients (slew rate 150 T/m/s, amplitude 30 mT/m) and a 5-element phased-array cardiac coil. Patients were placed on the CMR-table. A heating of the guiding catheter is in principle possible due to the metallic braiding. Therefore, the guiding catheter was removed to avoid any potential harm of the patients in the CMR scanner. The intracoronary infusion catheter was placed within the long sheath without the guiding catheter at the identical position as for measurement of coronary microvascular function. This identical position was fluoroscopically verified with the patient on the CMR table in the catheterization laboratory. The intracoronary infusion catheter as well as the long sheath were made without any metallic structures allowing their use in the CMR scanner. Patients were then moved to the CMR-scanner next to the catheterization laboratory. This novel study technique was established in 6 patients prior to inclusion of the study population.

Three short survey scans were performed to define the position and axis of the left ventricle. Resting left ventricular function was determined with cine images using a segmented k-space balanced fast-field-echo sequence (steady-state-free-precession) in short axis views in the true heart axis. The short axis scans covered the whole left and right ventricular chambers with 10–14 contiguous slices. Left ventricular ejection fraction and volume were determined using short axis volumetry (Easy Vision Software Rel. 5.1, Philips Medical Systems) as previously described (21). For first-pass-perfusion analysis a steady-state-free-precession sequence with three short axis slices was used with a saturation recovery pre-pulse before each slice. Repetition time was 2.4 ms. Echo time was 1.2 ms. Slice thickness was 10 mm. The field of view was adjusted between 360 mm and 410 mm with a rectangular reduction of 80% to 100% depending on the chest size of the patient (no fold-over tolerated). Measurement matrix was 115×128, resulting in a typical in plane resolution of 2.8×3.0 mm. With a similar resolution subendocardial from subepicardial perfusion could be discriminated (17). The parallel imaging technique SENSE was used with a reduction factor of two (22). Prospective triggering was

used for cardiac synchronization. The shortest trigger delay was chosen.

For perfusion analysis 0.025 mmol/kg body weight Gadolinium-DTPA (Schering Diagnostics, Berlin, Germany) was injected into a separate antecubital vein by a power injector (Medrad Spectris, Volkach, Germany) with 6 mL/s flushed with 20 mL of saline. This dosage was selected to avoid a possible saturation of the signal curve allowing a three-times application of the contrast agent for perfusion analysis. Patients were instructed to hold breath in end-expiration during first-pass. The patients' invasive blood pressure, oxygen saturation and electrocardiogram were continuously monitored (Millenia 3150 CMR, Invivo Research Incorporation, Pleasanton, CA, USA). CMR-perfusion studies were performed: after three-minutes intracoronary infusion of acetylcholine; after three-minutes intravenous infusion of adenosine in a dosage of 140 μg/min/kg body weight; and at rest. To study the endothelium-dependent first-pass perfusion in CMR patients received acetylcholine, which was applied through the intracoronary infusion catheter. The maximum acetylcholine concentration was chosen with less than 30% diameter luminal narrowing during prior measurement of coronary microvascular function in the catheterization laboratory. The three perfusion studies were performed at intervals of 15 minutes to allow equilibration of the contrast agent.

Quantitative myocardial perfusion analysis

We semi-quantitatively analyzed myocardial perfusion with the Easy Vision-5.2-Cardiac-MR-Prototype software (Release 1.2, Philips Medical Systems) twice by two independent observers unaware of endothelial function. Values were averaged. Each of the three slices of a perfusion study was analyzed in 6 segments (Fig. 1) as previously described (23), which resulted in 18 analyzed segments per patient. In each series of images the signal in the left ventricular blood pool and myocardium was measured. Every image was checked for optimal positioning of the selected region of interest with special regard to endocardial and epicardial borders. Adjustments were made for any respiratory movement. An index of myocardial perfusion for each segment was calculated by linear fitting of the myocardial upslope during first-pass contrast enhancement (17, 23). Myocardial upslope was normalized to the left ventricular blood-pool upslope to compensate for changes in the input function caused by the effects of the drug on heart rate and systemic circulation. Myocardial perfusion reserve index (MPRI) for adenosine was calculated as the ratio of the index of myocardial perfusion during adenosine stress to the index of myocardial perfusion at rest for every segment (23, 24). MPRI for acetylcholine was calculated in the same manner. For analysis of intravenously given adenosine all segments and for analysis of acetylcholine given via intracoronary infusion, segments 1 and 6 according to Fig. 1 were considered. For qualitative myocardial perfusion analysis subendocardial perfusion was visually estimated as normal or abnormal.

Biomarkers

Blood samples were taken under standardized conditions before cardiac catheterization and stored at −80°C. We measured serum values of the inflammatory markers soluble CD40 ligand (sCD40L), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and of the marker of endothelial activation soluble intercellular adhesion molecule-1 (sICAM-1) by ELISA-technique according to the manufacturers' instructions (R+D Systems, Wiesbaden, Germany, Bender MedSystems, Wien, Austria). Serum concentrations of

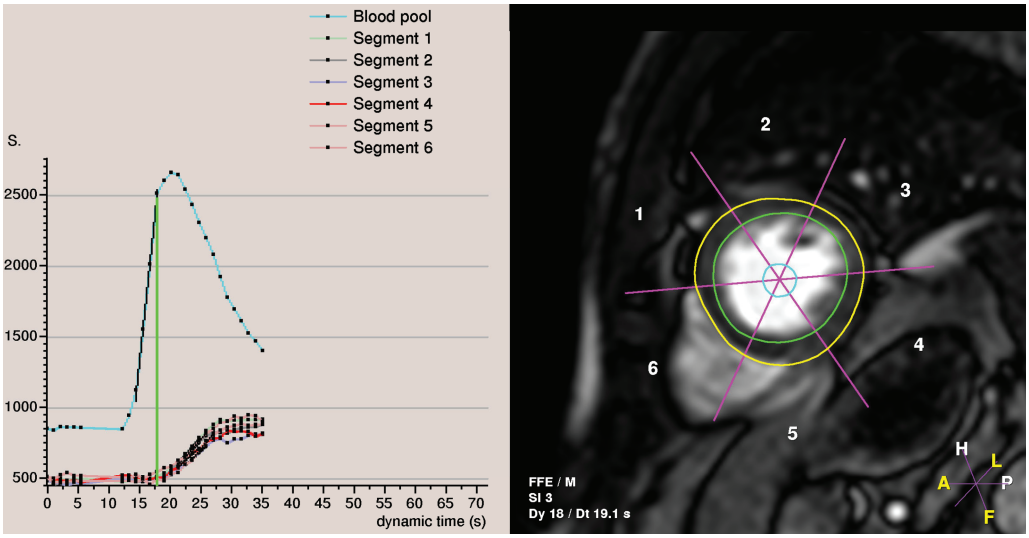

Figure 1. Quantitative analysis of myocardial perfusion in CMR. The right panel shows one dynamic scan of one slice with the segmentation into 6 equiangular segments starting clockwise from the anterior septal insertion point of the right ventricle. The left panel shows the upslope values for the left-ventricular blood-pool and for each of the 6 segments. The green bar indicates the position of the right image within the perfusion scan.

Table 1. Characteristics of patients

Patients	12
Men : Women	5 : 7
Age (yrs)	61.8 ± 8.2
Body mass index (kg/m^2)	26.1 ± 3.5
Cardiovascular risk factors (no.)	
Diabetes mellitus	0
Mild arterial hypertension	10
History of smoking	5
Hyperlipoproteinaemia	4
CMR parameters	
Enddiastolic volume (ml)	121 ± 34
Endsystolic volume (ml)	37 ± 17
Enddiastolic volume index (ml/m^2)	65 ± 16
Endsystolic volume index (ml/m^2)	20 ± 9
Cardiac output (l/min)	5.2 ± 0.9

C-reactive protein (CRP) were measured using high-sensitive-immunoradiometric assay (range 0.05 to 10 mg/L), as previously described (8). All measurements were performed in duplicate.

Statistics

There was no sample size calculation. Summary values are expressed as means ± standard deviation. Differences between means of continuous variables were analyzed using the t-test (Statistica version 6.0, StatSoft Inc., Tulsa, OK, USA). Multiple t-testing with Bonferroni correction were used to test for differences of MPRI between groups. Pearson's correlation coefficients (r) were calculated as Monte Carlo approximation to the exact test (StatXact version 6.0, Cytel Software Corporation, USA). Statistical significance was assigned at the $p < 0.05$ level.

RESULTS

We studied 12 patients without CAD but clinical symptoms of typical angina pectoris. Baseline characteristics are given in Table 1. All patients had normal left ventricular end-diastolic and end-systolic volumes, normal left ventricular muscle mass and cardiac output measured by CMR (Table 1). We were able to place the intracoronary infusion catheter at the fluoroscopically verified identical position in 8 of the 12 patients for CMR first-pass perfusion studies with endothelium-dependent stimulation. Data on measurements of adenosine are based on all 12 patients.

Table 3. Characteristics on measurement of coronary microvascular function

	Pulse pressure product	Coronary blood flow
Unit	mmHg × bpm	mL/min
Adenosine baseline	9510 ± 2977	37 ± 15
Adenosine stress	10959 ± 2491	105 ± 52
Acetylcholine baseline	9749 ± 2374	41 ± 16
Acetylcholine stress	11110 ± 2964	66 ± 31

bpm = beats per minute

Data referring to acetylcholine are based on 8 patients with successful placement of the intracoronary infusion catheter. Correlation coefficients for repeated measurements were 0.89–0.94 for quantitative coronary analysis, 0.99 for coronary blood flow velocity and 0.93 for quantitative myocardial perfusion analysis.

All serum levels of measured biomarkers were within normal range (Table 2). MPRI for adenosine was 1.48 ± 0.71, and MPRI for acetylcholine was 0.98 ± 0.39. We analyzed MPRI for adenosine and acetylcholine according to the median of the serum levels of each marker. MPRI was lower for patients with serum levels above the median compared to patients with serum levels below the median. We could show that this was evident for all measured biomarkers and for both, adenosine (Fig. 2A) as well as acetylcholine stimulation (Fig. 2B). Multivariable linear regression analysis for MPRI including all serum levels revealed that sCD40L (beta −0.55, $p < 0.001$) and TNF-α (beta −0.18, $p = 0.011$) were significantly associated with a decrease in MPRI on adenosine, as were TNF-α (beta −0.36, $p = 0.016$) and sICAM-1 (beta −0.49, $p = 0.022$) levels for a decrease in MPRI on acetylcholine.

An excellent Doppler flow signal was obtained in all 12 patients. Coronary blood flow and pulse-pressure-product significantly increased by intravenous application of adenosine as well as by intracoronary administration of acetylcholine ($p < 0.05$; Table 3).

Coronary blood flow reserve for endothelium-independent stimulation was 3.00 ± 1.15 and for endothelium-dependent stimulation 1.91 ± 1.15. Subendocardial perfusion was visually estimated as normal in 6 patients and as abnormal in the other 6 patients. Coronary blood flow reserves for adenosine (2.98 ± 0.89 versus 3.01 ± 1.55) and acetylcholine (1.56 ± 0.76 versus 2.27 ± 1.51) were lower in patients with abnormal

Table 2. Serum levels of markers of inflammation or endothelial activation

	Normal range	Mean	Standard deviation	Median	Lower tertile	Upper tertile
sCD40L (ng/mL)	0.03–3.98	8.43	4.52	6.84	6.71	11.70
TNF-α (pg/mL)	0–4.71	1.03	1.43	0.61	0.29	1.11
IL-6 (pg/mL)	0.43–9.96	3.72	2.71	3.35	1.99	4.24
sICAM-1 (ng/mL)	115–306	217.5	48.7	211.8	185.4	246.9
CRP (mg/L)	1–5	2.84	2.63	2.11	0.93	2.86

All serum levels of measured biomarkers were within normal range of the assay employed except sCD40L which was slightly elevated. Still, conflicting data exist on the normal range of this parameter in healthy subjects (26–28).

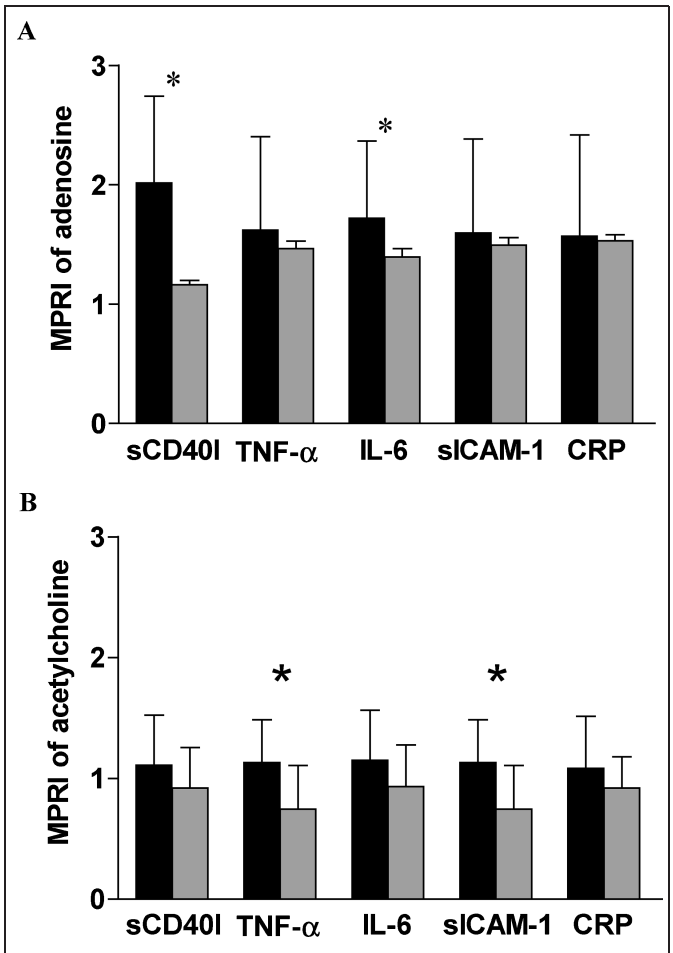

Figure 2. Black bars show myocardial perfusion reserve index (MPRI) for serum levels below the median. Grey bars show MPRI for serum levels above the median. (A) MPRI for endothelium-independent stimulation with intravenously applied adenosine. (B) MPRI for endothelium-dependent stimulation with acetylcholine given via intracoronary infusion. Statistical significance is indicated by $^{*}(p < 0.05)$. Adjustment for multiple testing was performed with the Bonferroni correction.

subendocardial perfusion compared with those exhibiting normal subendocardial perfusion. The myocardial perfusion reserves for adenosine (1.62 ± 0.89 versus 1.36 ± 0.45) and acetylcholine (1.04 ± 0.47 versus 0.93 ± 0.31) were higher in patients with normal subendocardial perfusion compared to those with abnormal subendocardial perfusion.

To examine whether coronary microvascular dysfunction may be a potential underlying mechanism for impaired MPRI, we correlated coronary blood flow reserve with MPRI. We found a significant correlation of MPRI with coronary blood flow reserve for both endothelium-independent stimulation with adenosine given intravenously ($r = 0.65$, $p = 0.022$) as well as for endothelium-dependent stimulation with acetylcholine by intracoronary infusion ($r = 0.75$, $p = 0.033$). In addition, MPRI for acetylcholine correlated significantly with MPRI for adenosine ($r = 0.78$, $p < 0.001$).

DISCUSSION

To our knowledge, this is the first study correlating myocardial first-pass perfusion in CMR with markers of early atherosclerosis such as coronary microvascular dysfunction and serum levels of biomarkers. We were able to demonstrate that in patients with typical angina pectoris without CAD even normal ranged serum levels of markers of inflammation or endothelial activation above the median were associated with an impaired myocardial perfusion reserve in CMR. This association was shown for both, endothelium-dependent as well as endothelium-independent stimulation of myocardial blood flow. Furthermore, our data revealed a significant correlation between myocardial perfusion reserve in CMR and coronary microvascular dysfunction.

Impaired endothelium-dependent vasodilation of the coronary microcirculation has been associated with exercise-induced myocardial ischemia in thallium-szintigraphy in patients without CAD (18). Due to its high spatial resolution first-pass CMR is superior to szintigraphic imaging techniques in detecting areas with reduced myocardial blood flow during pharmacological stress (20). Moreover, our approach to evaluate myocardial perfusion has previously been described as the best quantitative technique to assess myocardial perfusion with CMR in patients (17, 23). Recently, Panting et al. (17) revealed subendocardial hypoperfusion in syndrome X patients during intravenous administration of adenosine potentially representing an ischemic cause of chest pain. Our study extends this knowledge by showing that an impaired myocardial perfusion reserve index is significantly correlated with a decrease in coronary blood flow reserve for endothelium-dependent and endothelium-independent stimulation. These finding support the notion that myocardial hypoperfusion determined by CMR may be based on coronary microvascular dysfunction.

Since we could show a significant correlation of MPRI during endothelium-dependent and endothelium-independent stimulation, CMR combined with adenosine may represent a complete non-invasive tool to evaluate coronary microvascular dysfunction. This notion is bolstered by our data demonstrating that patients with visually abnormal subendocardial perfusion exhibit a lower coronary blood flow reserve and a lower MPRI for both endothelium-dependent and endothelium-independent stimulation.

We like to speculate about a potential prognostic value of an impaired myocardial perfusion reserve index in CMR during adenosine stress supporting its role as a potential novel study technique for coronary microvascular dysfunction. Our study shows that an impaired myocardial perfusion reserve index significantly correlated with coronary microvascular dysfunction. An impaired coronary microvascular function in patients without CAD has been shown to be associated with an increased risk of cardiovascular (2–4) and cerebrovascular events (5). Furthermore, in women without CAD, the presence of stress-induced reduction in myocardial phosphocreatine-adenosine triphosphate ratio by phosphorus-31 nuclear magnetic resonance spectroscopy, representing myocardial ischemia

was associated with an increased cardiovascular event rate during a three-years follow-up (25). Recent observations suggest that early atherogenesis is characterized by a low-grade inflammation altering endothelial function of coronary arteries. This is reflected by an increase in markers of inflammation or endothelial activation. The potential prognostic relevance of an impaired myocardial perfusion reserve index is strengthened by its association to serum levels of measured biomarkers. Of note, impaired MPRI was associated with normal ranged serum levels above the median. CRP levels and serum levels of other markers of inflammation and endothelial activation on the upper limit of the normal range have been associated with an increased rate of adverse events in patients without CAD (8, 12–16, 26). The correlation obtained between MPRI and invasively measured coronary flow changes and serum levels of biomarkers during acetylcholine or adenosine stimulation suggests that CMR is able to measure the perfusion effect of coronary microvascular dysfunction. Nevertheless, the prognostic impact of myocardial perfusion reserve index has to be addressed in a prospective study including a larger number of patients.

The major methodological novelty of our study is the combination of CMR perfusion with invasive intracoronary administration of acetylcholine allowing direct assessment of endothelial dependent vasomotor function at the myocardial level. To our knowledge, this is the first study reporting the use of an intracoronary catheter in patients during CMR perfusion analysis. To avoid any potential harm to patients due to metallic braiding of present available diagnostic or guiding catheters, we had to place the intracoronary infusion catheter only within the long sheath. With this technique, we were able to place the intracoronary infusion catheter for first-pass perfusion studies in CMR at the identical position as in prior measurement of coronary microvascular function in 8 of the 12 patients. There were no adverse effects, proving safety and feasibility of intracoronary acetylcholine administration during CMR perfusion analysis in such patients. For further studies on microvascular function and visualization of the coronary anatomy with cardiac CMR diagnostic or guiding catheters without any metallic structure need to be developed.

Study limitations

The limited number of patients enrolled in this safety and feasibility trial is a major limitation of this study. However, the novel study technique was established in 6 patients and all analyses were very carefully performed. This is indicated by calculation of coronary blood flow reserve based on a three-fold measurement of coronary blood flow velocity and three-fold quantitative coronary analysis. Furthermore, quantitative myocardial perfusion analysis in CMR and serum levels of biomarkers was based on the average of two complete measurements. In addition, exclusion of CAD was based on angiographic criteria. We did not use intravascular ultrasound to exclude angiographic not visible coronary atherosclerosis.

Conclusions

Our study demonstrates safety and feasibility of an intracoronary acetylcholine application during CMR perfusion analysis. We show that impaired myocardial perfusion reserve in magnetic resonance imaging is associated with established biomarkers of early atherosclerosis and significantly correlated with coronary microvascular dysfunction. Since levels of the biomarkers measured in this study have been linked to a substantial cardiovascular risk in patients without CAD (8–10), which has also been shown for coronary microvascular dysfunction (3, 5–7), our findings may stimulate further studies to determine the prognostic value of an impaired myocardial perfusion reserve index in CMR.

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