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Microcirculation function assessed by adenosine triphosphate stress myocardial contrast echocardiography and prognosis in patients with nonobstructive coronary artery disease

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Abstract

Background: Recent studies have demonstrated that coronary microcirculation dysfunction (CMVD) is closely correlated with adverse clinical outcomes. In this study, quantitative stress myocardial contrast echocardiography (MCE) was used to evaluate the CMVD and to investigate its association with the prognosis of patients with nonobstructive coronary artery disease (CAD).

Material and methods: From 2006 to 2014, 227 consecutive patients with chest pain and a diagnostic coronary angiography without significant coronary artery stenosis (<50%) who underwent adenosine triphosphate disodium (ATP) stress MCE were enrolled. Quantitative MCE measurements were analyzed using replenishment curves.

Results: Median follow-up time of this study was 5.3 years. Predictors of impaired coronary flow reserve (CFR) were smoking, diabetes, high apolipoprotein B, high low-density lipoprotein, serum uric acid, and low apolipoprotein A. During follow-up, 22 patients were reported to have 30 cardiac events (21 unstable angina, 3 nonfatal myocardial infarctions, 6 percutaneous coronary interventions). Using multivariate analysis, abnormal β reserve ($\leq$1.6), impaired CFR ($\leq$2.0), and diabetes were independent predictors of primary endpoint events in patients with nonobstructive CAD ($P < .05$). Multivariate analysis showed that CFR $\leq$2.0 (odds ratio [OR] = 25.21, 95% confidence interval [CI]: 3.01–182.32; $P = .003$), β reserve $\leq$1.6 (OR = 29.96, 95% CI: 3.5–241.27; $P = .002$), and diabetic (OR = 33.11, 95% CI: 3.65–300.02; $P = .002$) significantly increased the risk of the primary endpoint events.

Conclusions: ATP stress quantitative MCE is a feasible and effective method to evaluate microcirculation abnormalities in human coronary arteries and it can be used for the clinical analysis, risk stratification, and treatment of early CAD.

Abbreviations: ACE = angiotensin converting enzyme, ApoA = apolipoprotein A, ApoB = apolipoprotein B, ARB = angiotensin II receptor antagonist, ATP = adenosine triphosphate disodium, CAD = coronary artery disease, CFR = coronary flow reserve, CMVD = coronary microcirculation dysfunction, LDL-C = low density lipoprotein cholesterol, MACE = major adverse cardiac events, MBF = myocardial blood flow, MCE = myocardial contrast echocardiography, MI = myocardial infarction, PCI = percutaneous coronary intervention, ROI = regions of interest, SUA = serum uric acid.

Keywords: coronary flow reserve, coronary microcirculation, myocardial contrast echocardiography, prognostic value

1. Introduction

Recent studies have found that most acute coronary syndromes are more common in stable angina patients with nonobstructive coronary atherosclerosis (vulnerable) plaques. It can be assumed that acute coronary syndromes attribute to not only the vulnerable plaque but also the vulnerable myocardium microcirculation.[1] Therefore, there is a real need for an accurate diagnostic method to identify and treat patients with microcirculation dysfunction.

Currently, the golden standard test to diagnose coronary microcirculatory dysfunction (CMVD) is invasive coronary reactivity testing. However, only a small proportion of patients with angina eventually require revascularization. Therefore, a noninvasive method is needed to assess CMVD.

Myocardial contrast echocardiography (MCE) has been demonstrated to be an effective diagnostic algorithm for quantifying myocardial perfusion and measuring coronary flow reserve (CFR).[2–5] It has been reported that the myocardial blood flow (MBF) derived from MCE is correlated with the results of coronary Doppler flow measurements and human invasive CFR measurements.[6,7] Although the CFR threshold of 2.0 has been shown to accurately detect coronary artery disease (CAD),[3,8] the

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predictive value of CFR acquired through quantitative MCE testing is still unclear.

Adenosine triphosphate disodium (ATP), as a vasodilatory-stress agent, is used for pharmacological stress myocardial perfusion imaging as dipyridamole and adenosine.[9]

The purpose of this study was to use the quantitative ATP stress MCE to assess the CMVD and to investigate its association with the prognosis of nonobstructive CAD patients.

2. Material and methods

2.1. Patient population

This study was approved by the ethics committee of the First Affiliated Hospital of Harbin Medical University, and all the enrolled patients provided signed informed consent.

From 2006 to 2014, 227 consecutive patients presenting to Department of Cardiology of the First Affiliated Hospital of Harbin Medical University for chest pain were enrolled, met the inclusion and exclusion criteria, and performed ATP stress MCE. Inclusion criteria were epicardial coronary artery stenosis <50% demonstrated by coronary angiography and normal left ventricular systolic function (ejection fraction ≥50%). Exclusion criteria were history of myocardial infarction, percutaneous coronary revascularization, coronary artery bypass graft, cardiomyopathy, severe valvular disease, severe ventricular arrhythmias, presence of any intracardiac shunt, pulmonary embolism, anemia, and pulmonary disease.

2.2. Clinical and laboratory parameters included in analysis

CAD risk factors examined were age, gender, diabetes mellitus, hypertension, hypercholesterolemia, and previous or current smoking. Diabetes is determined by medical records and/or administration with oral hypoglycemic agents or insulin. Hypercholesterolaemia is defined as total cholesterol concentration ≥6.2 mmol/l or use of a cholesterol-lowering agent. Hypertension is defined as blood pressure ≥140/90 mm Hg or treatment with hypotensive agents. Smoking history (previous or current smoking). Blood samples were also tested for uric acid.

2.3. Study protocol

Images were obtained using Philips IE33 echocardiography system (Philips Ultrasound, Bothell, WA) and stored for subsequent analyses. MCE was performed in apical 2-, 3-, and 4-chamber views, the focus was set at the level of the mitral valve and frame rate was adjusted to 25 to 30 Hz. Ultrasound contrast agent SonoVue (Bracco Research SA, Geneva, Switzerland) was slow bolus injected (1.0–1.5 ml) followed by slow 5 ml saline flush over 20 seconds, repeated as necessary. Destruction-refill technique was used, after high mechanical index (MI = 1.7) "flash" impulse, replenishment was acquired at least 15 cardiac cycle by low MI (MI = 0.1) to evaluate the myocardial perfusion. The baseline contrast echocardiograms were obtained before ATP infusion and again after 3 minutes of ATP infusion. Application of liquid ATP, 20 mg/2 ml dosage form. ATP was infused through a syringe infusion pump to induce hyperemia, leading to an infusion rate of 160 ug/kg/min and a total infusion time of 6 minutes.[10]

Patients were monitored continuously by blood pressure and electrocardiography before, during, and 20 minutes after ATP infusion. To avoid interaction, the interval between MCE and coronary angiography was 3 to 5 days.

2.4. Quantitative analysis of MCE

Quantification was performed using QLAB version 7.1 (Phillips Medical Systems, Best, The Netherlands), quantification of myocardial perfusion with 17 myocardial segments in apical 2-, 3-, and 4-chamber views.[11] The regions of interest (ROI) was placed in myocardial segment at end-systole, not to include structures such as the left ventricular cavity and pericardium. The software automatically calculated myocardial plateau signal intensity (A) and signal intensity exchange rate (β) of each ROI according to the exponential curve fitting formula: $y = A \times (1 - e^{-\beta t})$, A reflects the myocardial blood volume, and β reflects the myocardial blood velocity.[12] The $A \times \beta$ represents the MBF. CFR equals stress divided by rest MBF.

2.5. Long-term follow-up

Clinical long-term follow-up was performed through examination of hospital records and telephone follow-up. Primary events comprised the occurrence of unstable angina, nonfatal myocardial infarction, and percutaneous coronary interventions. The follow-up time was defined as the date of the event; if no event occurred, the date of the last telephone follow-up or hospital records was defined as the follow-up time.

2.6. Statistical analysis

Measurement data of normal distribution were described by mean ± standard deviation, t test was used for comparison between groups, and measurement data with non-normal distribution were described by median (P25, P75), nonparametric test was used for comparison between groups. The count data is described by the number of cases and the composition ratio or rate, the comparison between groups is performed by χ^2 test; the nonconditional multivariate logistic regression was used to analyze the influencing factors of the primary endpoint and the forward condition was used to screen the independent variables. Kaplan–Meier survival curves were performed to evaluate the distribution of time to the primary endpoint events. ROC curves were performed to test the sensitivity, specificity and the area under the curve (AUC) of reserve β.

All statistical analyses were performed using SPSS version 17.0 (SPSS Inc). P-value <.05 was considered statistically significant.

3. Results

Follow-up was performed on 227 patients. 89 patients were excluded because of lost to follow-up and the present study population consisted of 138 patients (56.20 ± 10.57 years, 60 men [43%]). A mean follow up time of 5.3 years. Most patients (78%) had ≥1 risk factor for CAD.

The heart rate at peak stress increased significantly compared to baseline (89 ± 16 beats/min vs 64 ± 15 beats/min, $P < .001$). There was no significant difference between systolic blood pressure (130 ± 20 vs 125 ± 15 mm Hg; $P = .024$) and diastolic blood pressure (80 ± 12 vs 75 ± 14 mm Hg; $P = .041$) at baseline and peak stress.

Table 1

Patients characteristics according to coronary flow reserve level.

Variable	Stress MCE		*P*-value
	CFR >2.0 n=118 (86%)	CFR ≤2.0 n=20 (14%)	
Demographics			
Age, yr	56.65±10.89	58.19±7.66	.203
Gender, n (%)			.842
Male	50 (42.4)	8 (40)	
Female	68 (57.6)	12 (60)	
Risk factors			
Hypertension, n (%)			.147
No	62 (52.5)	7 (35)	
Yes	56 (47.5)	13 (65)	
Diabetes mellitus, n (%)			<.001*
No	100 (84.7)	2 (10)	
Impaired glucose tolerance	3 (2.5)	6 (30)	
Diabetes mellitus	15 (12.7)	12 (60)	
Smoking history, n (%)			.006*
No	79 (66.9)	7 (35)	
Yes	39 (33.1)	13 (65)	
Dyslipidemia, n (%)			.022*
No	56 (47.5)	4 (20)	
Yes	62 (52.5)	16 (80)	
Total cholesterol, mmol/l	5.48±1.46	5.72±1.26	.077
Triglyceride, mmol/l, median (P25, P75)	1.75 (1.12, 2.52)	1.91 (1.34, 2.81)	.083
High-density lipoprotein, mmol/l	1.33±0.46	1.18±0.16	.131
Apolipoprotein B, mmol/l, median (P25, P75)	1.01 (0.77, 1.32)	1.18 (0.82, 1.54)	.021*
Lipoprotein a, mmol/l, median (P25, P75)	21.80 (14.90, 38.85)	22.95 (14.29, 32.53)	.091
Apolipoprotein A, mmol/l, median (P25, P75)	1.28 (1.10, 1.47)	1.19 (1.01, 1.33)	.020*
Low-density lipoprotein, mmol/l	3.59±0.45	3.77±0.65	.011*
Fasting blood-glucose, mmol/l, median (P25, P75)	5.32 (4.7, 6.2)	5.47 (5.01, 6.32)	.387
Uric acid, umol/l, median (P25, P75)	300.48 (252.11, 389.07)	356.44 (300.23, 392.22)	.000*
Medications, n (%)			
Aspirin			.136
No	19 (16)	6 (30)	
Yes	99 (84)	14 (70)	
ADP receptor antagonist			.536
No	50 (42)	7 (35)	
Yes	68 (58)	13 (65)	
Lipid-lowering drugs			.248
No	17 (14)	1 (5)	
Yes	101 (86)	19 (95)	
ACEI/ARB			.572
No	67 (57)	10 (50)	
Yes	51 (43)	10 (50)	

ACE=angiotensin converting enzyme, ADP=adenosine diphosphate, ARB=angiotensin II receptor antagonist, CFR=coronary flow reserve.
* $P<.05$.

3.1. Quantitative MCE

Quantitative analysis of MCE was performed in 138 patients. Quantitative MCE can be used to analyze 1806 (77%) segments at rest, 1853 (79%) segments at stress, and 1689 (72%) segments for reserve measurements.

Failure of curve fitting, artifacts, and attenuation resulted in unable to perform quantitative MCE analysis on some segments.

3.2. Myocardial perfusion in relation to clinical and laboratory markers

Divide patients into 2 groups using CFR cut-off point 2.0.[13] The baseline characteristics of the populations according to their response to ATP are shown in Table 1. Patients with impaired CFR had a higher prevalence of diabetes mellitus ($P=<.001$) and smoking history ($P=.006$). Patients with low CFR also had significantly higher levels of apolipoprotein B (apoB) ($P=.021$), low-density lipoprotein cholesterol (LDL-C) ($P=.011$), serum uric acid (SUA) ($P<.001$) and a lower apolipoprotein A (apoA) ($P=.020$). There was no statistically significant difference in the frequency of prior use of aspirin, lipid-lowering drugs, adenosine diphosphate receptor antagonist, or angiotensin-converting enzymes inhibitors/angiotensin receptor blocker among patients in 3 groups.

3.3. ROC curve

ROC curve of reserve β provided an accurate method for the prediction of primary endpoints by MCE in nonobstructive CAD patients. Reserve β cut-off 1.6 provided the best prediction, with

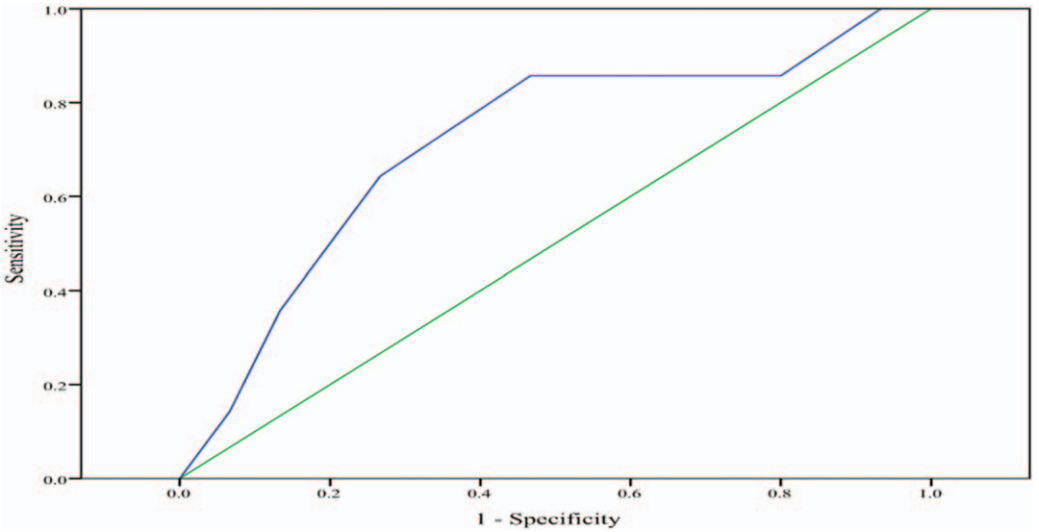

Figure 1. Receiver operating curve of reserve β under ATP stress MCE in the prediction of primary endpoints. AUC value for reserve β is 0.71. AUC = area under the curve, ATP = adenosine triphosphate disodium, MCE = myocardial contrast echocardiography.

Table 2

Cardiac events at follow-up.

	CFR >2.0 (n=118; 86%)	CFR ⩽2.0* (n=20; 14%)
Unstable angina, n	12	9
Nonfatal MI, n	0	3
PCI, n	0	6

CFR = coronary flow reserve, MI = myocardial infarction, PCI = percutaneous coronary interventions.
* $P < .05$ versus CFR > 2.0 group.

67% sensitivity and 73% specificity (AUC 71, 95% confidence interval, 47–95) for major adverse outcomes (Fig. 1).

3.4. Predictors of events

During follow-up, a total of 22 patients (16%) had cardiac events and 116 patients without primary endpoints. Twenty-two patients (8 patients in preserved CFR group, 14 patients in impaired CFR group) developed 30 cardiac events (21 unstable angina, 3 nonfatal myocardial infarction, 6 percutaneous coronary interventions) (Table 2). Figure 2 illustrates Kaplan–Meier survival curves according to cut-off value for CFR of 2.0

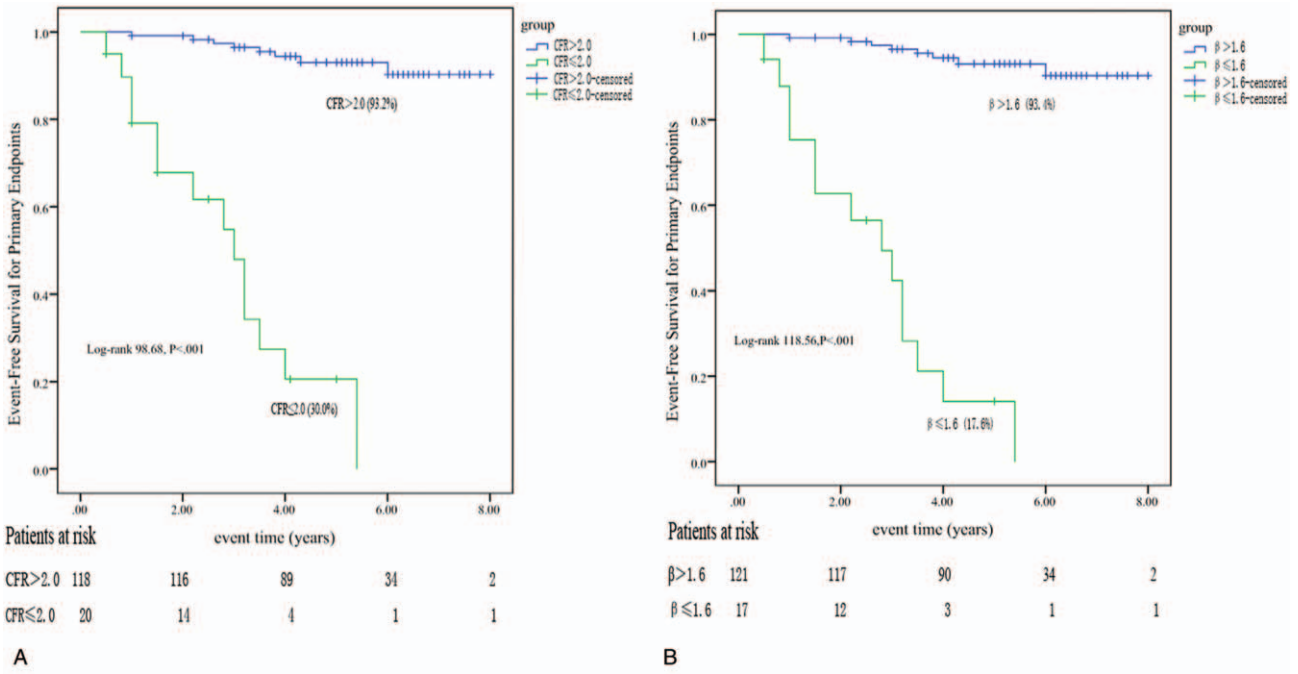

Figure 2. Kaplan–Meier survival curves of patients according to the cutoff value of CFR (A) and β reserve (B). CFR = coronary flow reserve.

Table 3

Quantitative myocardial perfusion parameters at baseline, hyperemia, and reserve in patients with or without primary endpoints.

Parameters of MCE	Primary endpoints (n = 22; 16%)			Without primary endpoints (n = 116; 84%)		
	Baseline	Hyperaemia[†,*]	Reserve[*]	Baseline	Hyperaemia[‡]	Reserve
B, s^{-1}	0.65 ± 0.23	0.98 ± 0.45	1.51 ± 0.40	0.68 ± 0.25	1.77 ± 0.68	2.61 ± 1.07
MBF, dB/s	9.11 ± 3.10	21.22 ± 5.22	2.23 ± 1.88	10.21 ± 0.42	31.65 ± 6.15	3.11 ± 2.05

MBF = myocardial blood flow, MCE = myocardial contrast echocardiography.
[*] Compared with patients without primary endpoints, $P < .001$.
[†,‡] Compared with baseline, $P < .001$.

and β of 1.6 for the prediction of major adverse outcomes. Patients with primary endpoints events were older (61.71 ± 7.56 vs 55.23 ± 10.92 years) ($P = .02$) and had significantly more history of diabetes mellitus (47.3% vs 9.0%) ($P < .001$) , hypertension (64.7% vs 39.7%) ($P = .004$), and smoking (59.8% vs 29.4%) ($P = .016$) versus those without primary endpoints events.

Baseline perfusion parameters-β and MBF, significantly increased during hyperemia for both patients with and without primary endpoints. Peak hyperemia and reserve parameters of β and MBF were significantly impaired in patients with primary endpoints compared with patients without primary endpoints (Table 3).

Take the occurrence of the primary endpoint events as the dependent variable, significance indicators in univariate analysis, age, history of diabetes and hypertension, smoking history, reduced β reserve and CFR abnormalities as independent variables, the conditional forward method was used to gradually screen for independent variables. The results showed that reduced β reserve and impaired CFR, diabetes were independent risk factors for the primary endpoint events occurred ($P < .05$). Compared with the preserved CFR (>2.0), the impaired CFR (≤2.0) was associated with 25.21-fold increased risk for primary endpoint events. A β reserve ≤1.6 had 29.96-fold increase in primary endpoint events compared with patients with β reserve >1.6. The risk of primary endpoint events in patients with diabetes was 33.11 times that of patients without diabetes, as shown in Table 4.

4. Discussion

CMVD causes myocardial ischemia leading to angina[14,15] and is associated with major adverse cardiac events (MACE).[13,16] The current study demonstrated that quantitative MCE was an effective technique for detecting myocardial perfusion abnormalities in patients with nonobstructive CAD during ATP stress. Quantitative parameters β reserve and CFR can be used for risk stratification and determine the prognosis prediction.

4.1. Risk factors affecting coronary microvasculature

A previous study demonstrated that women with persistent chest pain exhibited a higher incidence of nonobstructive CAD compared to men.[17] Nevertheless, other studies have reported no significant differences in the prevalence of CMVD between men and women.[16,18] In this study, we found that gender was not significant correlated with impaired CFR. In the present study, reduced CFR was associated with diabetes mellitus, smoking, and dyslipidemia. The iPOWER study[19] showed that low CFR was only associated with high-density lipoprotein cholesterol, but not with other serum lipids. However, our findings showed that CFR was associated with higher levels of apoB and elevated LDL-C, as well as lower levels of apoA. The findings of our study are in line with other studies.[20–22] Moreover, we found that reduced CFR was associated with higher levels of SUA. This is consistent with previous studies,[23] which revealed that patients with microvascular angina had higher levels of SUA and that SUA levels predicted carotid atherosclerosis. We observed that hypertension was relatively more common in the CFR ≤2.0 group, although there was no significant difference in the incidence of hypertension between the groups.

Lavi et al[24] found that smokers without significant CAD had abnormal epicardial endothelial function, while coronary microvascular endothelial function remained normal. Another study demonstrated that among women with CMVD, conventional

Table 4

Multivariate logistic regression analysis of influencing factors of primary endpoints.

Variable	β value	$S_{\bar{x}}$	Wald χ^2 value	*P*-value	OR value (95%CI)
Gender				.800	
Age, yr				.020	
CFR					
>2.0					1.000
≤2.0	3.01	1.10	8.53	.003[*]	25.21 (3.01–182.32)
β reserve					
>1.6					1.000
⩽1.6	3.24	1.04	9.22	.002[*]	29.96 (3.5–241.27)
Diabetes mellitus					
no					1.000
Impaired glucose tolerance	2.36	1.27	3.43	.064	10.68 (0.87–130.64)
Diabetes mellitus	3.50	1.12	9.69	.002[*]	33.11 (3.65–300.02)

CFR = coronary flow reserve, CI = confidence interval, OR = odds ratio.
[*] $P < .05$.

cardiovascular risk factors accounted for <20% of observed variability in response to adenosine.[25] Therefore, the difference between the findings may be due to different sensitivities to traditional cardiovascular risk factors, between the epicardial system and the coronary microcirculation.[18]

4.2. Predictive value of quantitative MCE for clinical events

In a recent study, positron emission tomography myocardial perfusion imaging was performed to evaluate CMVD among patients without visual evidence of CAD, showing that CMVD was a strong predictor of adverse cardiovascular events with a hazard ratio of 0.8 per 10% increase in CFR.[16] Thus, noninvasive assessment of CMVD may be an important method of risk-stratifying these patients.

Rinkevich and colleagues reported that resting β and MBF were increased in cardiac syndrome X.[26] Hansen et al demonstrated the existence of impaired resting MBF analyzed by quantitative MCE in type 1 diabetic patients.[27] On the contrary, we found no significant difference in quantitative parameters at rest. The possible reason is that previous studies enrolled healthy volunteers as control group, while the majority of patients included in the present study had at least one cardiovascular risk factor.

Accurate risk stratification of nonobstructive CAD patients can provide important information to guide clinical management. This study suggested that quantitative MCE had the potential to identify the risk of acute coronary syndrome. Our study extends previous findings in which impaired CFR predicted increased mortality[28] and improves prediction of major adverse outcomes over angiographic and risk factors[29] in patients with suspected CAD, supporting impaired CFR ($\leqslant$2.0) was an independent predictor of primary endpoint events in patients with non-obstructive CAD. Compared with preserved CFR (>2.0), the incidence of MACE increased by 25.21 times in patients with impaired CFR ($\leqslant$2.0). Furthermore, we found abnormal β reserve ($\leq$1.6) and diabetes mellitus were independent risk factors for the prognosis of nonobstructive CAD patients. A β reserve $\leq$1.6 had a 29.96-fold increase in MACE compared with patients with β reserve >1.6. Independent prediction of abnormal β reserve indicated that in the distribution of impaired CFR, high-risk patients can be further identified.

The present study demonstrates that it is feasible to apply quantitative MCE in patients with nonobstructive CAD, and the results are similar to those found with invasive assessment.

4.3. Study limitations

The major limitation of our study was that we did not evaluate the control group without chest pain, so we were not able to identify whether the abnormalities we found were related to the patient's chest pain.

Furthermore, failure of refilling curve fitting, artifacts or attenuation (usually observed in base-middle anterior and basal anterolateral wall segments) resulted in 28% of segments failing to quantify the reserve parameters by MCE.

Finally, we did not evaluate how left ventricular function behaved over time in patients with reduced or preserved CFR. Studies have shown that CMVD might be one of the underlying mechanisms of dilated cardiomyopathy. In this study, we studied a selected population of patients with preserved ventricular function (left ventricular ejection fraction $\geq$50%) and ruled out severe heart disease. After long-term follow-up, there was no significant change in systolic and diastolic function in all patients, although a small proportion of patients had reduced diastolic function.

5. Conclusions

In conclusion, quantitative ATP stress testing based on β reserve and CFR measurements derived from MCE is a feasible method for the noninvasive and reliable assessment of abnormalities of coronary microcirculation in humans, and can help in the clinical analysis, risk assessment, and treatment of CAD patients.

Author contributions

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Writing – review and editing: Wei Han.

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