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Elevated serum endothelin-1 is an independent predictor of coronary microvascular dysfunction in non-obstructive territories in patients with coronary artery disease

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ABSTRACT

Objective: Endothelin-1 contributes to the constrictor response of the coronary arteries in patients with ischemia with normal coronary arteries. There is thus increasing evidence that endothelin-1 plays a role in coronary microvascular dysfunction (CMD). We investigated whether elevated endothelin-1 is associated with CMD in patients with coronary artery disease (CAD).

Methods and Results: We prospectively studied 49 consecutive CAD patients with 1- or 2-vessel disease (age 71 ± 10 yrs, 43 males). Myocardial blood flow (MBF) was measured by ^{15}O-water PET/CT at rest and during stress, and the coronary flow reserve (CFR) was calculated by dividing the stress MBF by the rest MBF. A CFR of less than 2.0 in non-obstructive regions was defined as a marker of CMD. Eighteen out of 49 (37%) CAD patients had CMD. Endothelin-1 in patients with CMD was significantly higher than in those without CMD (2.27 $\pm$ 0.81 vs. 1.64 $\pm$ 0.48 pg/mL, P = 0.001). Accordingly, univariate ROC analysis showed that the continuous endothelin-1 levels significantly discriminated between the presence and absence of CMD (area under the curve = 0.746 [95%CI 0.592–0.899]). The dichotomous treatment of elevated endothelin-1 as

1.961 pg/mL or more yielded the optimal discriminatory capacity, with a sensitivity of 72.2% and a specificity of 71.0%. High endothelin-1 was still a significant predictor of CMD after adjusting for diabetes mellitus (odds ratio = 6.64 [1.75–25.22], P = 0.005).

Conclusion: Endothelin-1 is associated with CMD in non-obstructive territories in patients with CAD, suggesting that endothelin-1 is a potential target for treating CMD in CAD patients.

Keywords

Endothelin-1, coronary microvascular dysfunction, coronary artery disease

Sub-headings

Serum endothelin-1 and coronary microvascular dysfunction

Introduction

In patients with coronary artery disease (CAD), a coronary flow reserve (CFR) of less than 2.0 in non-obstructive territories should be diagnosed as coronary microvascular dysfunction (CMD).[1] This is a very important issue because the maximal value of CFR after revascularization in obstructive territories is expected to be the level of CFR in remote non-obstructive areas. Accordingly, the contribution of CMD to the reduced CFR should be a focus of investigation in the revascularization era.

Endothelin-1 is a significant vasoconstrictor.[2] The production of endothelin-1 has been shown to correlate with the constrictor response of the coronary diameter, and serum endothelin-1 was demonstrated to be associated with coronary vascular resistance in patients with chest pain and normal coronary arteriograms.[3][4] There is thus increasing evidence that endothelin-1 plays a role in coronary microvascular dysfunction (CMD).[4][5]

Although flow-limiting coronary stenosis is a major predictor of reduced CFR in patients with CAD, CFR can also be reduced in non-obstructive areas, which mainly

indicates the presence of CMD. The pathophysiological significance of serum endothelin-1 in CMD can be more precisely assessed by using quantitative CFR.

Coronary revascularization can relieve the flow-limiting stenosis, but cannot ameliorate CMD.[6] The optimal medical therapies for CAD, such as exercise[7], statins[8], and ranolazine,[9] have been shown to ameliorate CMD in part. The noninvasive quantitative measurement of myocardial blood flow (MBF) and CFR by oxygen-15 labeled (^{15}O-) water positron emission tomography (PET) and adenosine triphosphate (ATP)-induced hyperemia is useful for the detection of CMD in patients with non-obstructive CAD.[6] We therefore designed the present prospective study to assess the contribution of serum endothelin-1 to CMD in non-obstructive regions in patients with CAD.

Materials and Methods

Study Population

Eighty-two consecutive patients with obstructive CAD who underwent ^{15}O-water PET/computed tomography (CT) between July 2015 and April 2017 at Hokkaido University Hospital were prospectively enrolled in this study. After excluding 1 patient due to an incomplete PET scan and 4 patients due to the absence of blood samples, we collected data on the remaining 77 patients with CAD. We then excluded 27 patients who had 3-vessel disease (VD) with or without left main artery (LM) disease and 1 patient with LM disease plus right coronary artery (RCA) disease, and the data on the remaining 49 patients with 1-VD or 2-VD (43 males and 6 females; age 71 ± 10 [SD] years) were entered into the analysis (Fig. 1). There was no patient with only LM disease. Patients with a history or clinical evidence of bronchial asthma were excluded from the study.

All of the subjects refrained from caffeine-containing beverages for at least 24 hr before the PET study. Informed consent was obtained from each study subject. The study protocol was approved by the ethics committee of each institution and

registered with the University Hospital Medical Information Network clinical trials registry (no. UMIN000018160), and the procedures were in accordance with institutional guidelines and the principles of the Declaration of Helsinki. The primary outcome of this prospective study was to assess the effects of coronary revascularization on CFR, and the findings were recently published.[6]

Blood Chemical Analysis

Venous blood samples were obtained at the same time as the PET scans. They were centrifuged at 4°C at 2,500 rpm for 15 min, and their supernatant was stored at −70°C. An enzyme-linked immunosorbent assay (ELISA; Bio-Techne) was used to measure the levels of serum endothelin-1 in duplicate.

PET Scans

All PET/CT scans were performed using a Gemini TF PET/CT scanner (Philips Healthcare, Cleveland, OH) with a 64-row-detector CT system. PET was performed as previously described.[10] After a low-dose CT scan at free-breathing for attenuation and scatter correction, 500 MBq of ^{15}O-water was administered to the subject intravenously over a

100-sec period with a simultaneous 6-min list-mode acquisition. All PET/CT scans were executed in 3D mode.

Pharmacological stress was induced by an intravenous injection of ATP (160 μg/kg/min) at 3 min before the emission scanning and was continued for a total of 9 min. The subject's heart rate and blood pressure were recorded before and at 1-min intervals during the ATP infusion. Before the attenuation correction, manual registration was performed in the coronal, sagittal, and transaxial views. The rest and stress PET/CT images were visually aligned for proper registration, carefully ensuring that the left ventricular myocardial activity on PET did not overlap with the lung parenchyma on CT.[11] The list-mode data were subdivided into 24 serial frames (18 × 10 and 6 × 30 sec). Attenuation-corrected radioactivity images were reconstructed using a 3D-row action maximum-likelihood algorithm (iterations, 2; relaxation parameter, 0.012). Heart rate, blood pressure, and a 12-lead electrocardiogram were recorded at rest and at 1-min intervals during and after the administration of ATP. The estimated radiation exposure per examination was 4.2 mSv, including <0.1 mSv from the scout scan, 1.2 mSv from the CT performed for coronary artery calcium scoring, 0.7 mSv from the CT performed for attenuation correction, and 1.1 mSv for each PET scan. Each of the 17 PET myocardial segments was assigned to

the corresponding coronary vessel by using a standard 17-segment model.

Quantification of MBF

We quantified MBF using a software program developed in-house that semi-automatically defines regions of interest for the left ventricular blood pool and the left ventricular myocardium. The MBF was calculated using a one-tissue-compartment tracer kinetic model, including a myocardium-to-blood spillover correction.[10][11] The CFR was calculated as the ratio of the MBF during stress to the MBF at rest. The inter-observer variability of MBF and CFR was excellent.[10]

Coronary Angiography

All patients underwent selective coronary angiography with the use of standard clinical techniques. To assess the per-patient angiographic disease burden, significant CAD was identified when the stenosis of a coronary artery was ≥50% by visual assessment on invasive coronary angiography.

Definition of CMD in Non-obstructive Territories

If patients were diagnosed with 1-VD, 2-VD, or LM disease without RCA disease, then non-obstructive territories were recognized. For example, in a patient with 1-VD of RCA disease, the left anterior descending artery (LAD) and left circumflex (LCx) regions were non-obstructive. Then, the average CFRs in the LAD and LCx territories were calculated as $CFR_{\_remote}$. If CFR_{remote} was less than 2.0, the patient was defined as having CMD.[12]

Statistical Analyses

Continuous variables are presented as means ± SD. Categorical variables are presented as absolute numbers with percentages. Differences between groups were evaluated using the Wilcoxon rank-sum test followed by the Steel-Dwass test for continuous data and Fisher's exact test for categorical data. We performed a univariate and multiple logistic regression analysis to examine the association between the presence of CMD and each of serum endothelin-1 (binary), age, gender, history of smoking, hypertension, diabetes, dyslipidemia, and history of myocardial infarction. A p-value <0.05 was considered significant. All statistical analyses were performed using STATA

12.1 (StataCorp, College Station, TX).

Results

Clinical Characteristics of the Study Subjects

The global CFR in patients with 3-VD (n = 23) and LM with 3-VD (n = 5) was significantly lower than that in patients who had non-obstructive remote areas such as 1-VD (n = 22), 2-VD (n = 26), and LM disease without RCA disease (n = 1) (Fig. 1). CMD was observed in 18 patients out of 49 (37%). Table 1 summarizes the clinical characteristics of the presence and absence of CMD in non-obstructive regions defined as reduced CFR (< 2.0) and normal CFR (≥ 2.0), respectively. The patients with CMD had high global rest MBF, low global stress MBF, low global CFR, and high serum endothelin-1. In a regression analysis, serum endothelin-1 was not directly associated with global rest MBF (r = 0.063, P = 0.208), but was directly associated with global stress MBF (r = - 0.264, P = 0.016) and global CFR (r = - 0.501, P = 0.001).

Receiver Operating Curve (ROC) Analysis for the Detection of CMD by Endothelin-1

Mean endothelin-1 did not differ based on anatomical disease severity (LM disease/3-VD: 1.73 ± 0.59 vs. 1- to 2-VD: 1.87 ± 0.69 pg/mL, P = 0.35). However, endothelin-1 was significantly higher in patients with CMD than in those without CMD (Fig. 2).

Univariate ROC analysis showed that the continuous endothelin-1 levels significantly discriminated between the presence and absence of CMD (area under the curve = 0.746 [95%CI 0.592–0.899]) (Fig. 3). The threshold value of 1.961 pg/mL or more yielded the optimal discriminatory capacity, with a sensitivity of 72.2% and a specificity of 71.0%. Accordingly, the rate of CMD in patients with a high endothelin-1 level (≧1.961 pg/mL) was higher than that in patients with a low endothelin-1 level (<1.961 pg/mL) (Fig. 4).

Relationship between CFR and Clinical Variables

In a univariate logistic analysis, the CMD in non-obstructive territories was significantly associated with serum endothelin-1, but not with baseline characteristics such as age, gender, smoking, diabetes, hypertension, dyslipidemia, and history of myocardial infarction (Table 2).

A multivariate analysis including endothelin-1 category and diabetes revealed that endothelin-1 was a significant independent predictor of CMD (Table 2).

Discussion

Using ^{15}O-water PET, we demonstrated that CMD, as defined by low CFR (< 2.0) in non-obstructive territories, was observed in 37% of patients with 1-VD, 2-VD, and LM disease without RCA disease. Second, a high serum endothelin-1 level of 1.961 pg/mL or more could discriminate the CMD in non-obstructive territories, while endothelin-1 was not associated with the anatomical disease burden (3-VD/LM disease vs. 1-VD and 2-VD). These findings shed light on CMD in the non-obstructive territories as another important risk factor in addition to flow-limiting coronary artery stenosis, and suggest that endothelin-1 is a potential target for treating CMD as a residual risk factor in patients with CAD.

Elevated Serum Endothelin-1 and CMD

A recent report from the Coronary Vasomotion Disorders International Study Group (COVADIS) shows the international standardization of diagnostic criteria for microvascular angina, in which impaired CFR is one of the clinical criteria for evidence of impaired microvascular function.[13] In our present study we also used a CFR assessed by dedicated PET, which provided regional and global CFR in detail.

Accordingly, per-vessel CFR assessed by PET successfully revealed the presence of CMD in the non-obstructive myocardium, along with the relationship between endothelin-1 and CMD. This result has extended the previous studies showing that endothelin-1 is significantly associated with CMD in areas of myocardium subtended by non-obstructed coronary arteries.[4][5]

There are two forms of endothelin, endothelin-1 and endothelin-2, both of which are small, biologically active peptides, and there are two receptors for endothelin, endothelin-A and -B.[2] Endothelin-1 mediates activation of the G protein-coupled endothelin-A receptor on vascular smooth muscle cells and induces endothelial dysfunction, inflammation, and vasoproliferative effects, which in turn induce coronary vasoconstriction.[14][15][16] A recent study showed that the chronic elevation of circulating endothelin-1 in microvascular angina is associated with genetic factors such as the rs9349379-G allele status and is a potential therapeutic target in patients with microvascular angina.[15] Elevated endothelin-1 is implicated in systemic hypertension, pulmonary hypertension, chest pain with normal coronary angiogram, kidney disease, heart failure, and CAD.[3][17][18][19] In addition, the effects of endothelin-1 on

atherosclerotic burden have been well studied.[2][17] The results of these previous investigations demonstrated that elevated endothelin-1 in CAD patients was not associated with disease burden, but was a significant predictor of cardiac events.[16][17] Our study similarly showed that the endothelin-1 concentration was related to CMD, but not to the CAD burden. In the present study, patients without nonobstructive areas were excluded because the presence of ischemia with obstructive coronary stenosis is different from the presence of CMD. These results suggest that the association of CMD with high endothelin-1 shown in our study may explain the poor outcomes in patients with high endothelin-1 levels.

Another important finding of our study is that serum endothelin-1 was not directly associated with global rest MBF, but was directly associated with global stress MBF and global CFR. This result suggests that endothelin-1 is a powerful vasoconstrictor affecting the coronary circulation mainly during stress in patients with CAD. In the future, the effects of an endothelin-A antagonist on CAD with CMD could be quantitatively confirmed by measuring CFR and rest and stress MBF.

Clinical Perspective of Endothelin-1 in CMD

In the revascularization era, it has become possible to revascularize flow-limiting coronary stenosis in patients with CAD. However, 42% of patients who undergo coronary revascularization still have low CFR due to concomitant CMD, even if they receive the optimal medical therapy based on the guidelines.[6] This result suggests that drugs to improve CFR such as statins,[8] beta-blockers,[20] and angiotensin receptor blockers [21][22] are of limited value for resolving CMD up to the normal levels in patients with CAD and CMD. Accordingly, several studies have tested the effects of drugs such as ranolazine and endothelin A-receptor blockade on CFR.[9][23] These studies, together with our present experiments, suggest that endothelin-1 might be a specific and significant therapeutic target for improving CMD. In fact, a prospective study on therapeutic manipulation of endothelin-1 in microvascular angina is currently underway.[24] The study tests the efficacy and safety of adjunctive treatment with an oral selective inhibitor of the endothelin-A receptor in patients with microvascular angina.

Study Limitations

Several study limitations should be noted. First, no outcome information was provided. Second, the number of patients who had non-obstructive areas (n=49) was too small for us to conduct a fully-adjusted analysis using clinical variables; we therefore entered the presence of DM as a significant influencer of CMD in general and a significant univariate predictor (i.e., binary of endothelin-1) into a multivariate analysis.

Conclusions

Elevated serum endothelin-1 is a major independent predictor of CMD as measured by ^{15}O-water PET with ATP infusion in patients with obstructive CAD. The present results provide evidence that increased endothelin-1 might be a therapeutic target for CMD in non-obstructive territories.

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Conflict of interest

All other authors report that they have no relationships relevant to the contents of this paper to disclose.

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Figure Legends

Figure 1. Study population. CAD = coronary artery disease, CFR = coronary flow reserve, VD = vessel disease, LM = left main artery, RCA = right coronary artery.

Figure 2. Comparison of serum endothelin-1 in patients with CMD in the non-obstructive territories (green bar) and without (orange bar). Vertical bars represent the mean with standard deviation. CMD = coronary microvascular dysfunction in the non-obstructive territories.

Figure 3. Univariate ROC analysis to detect the presence of CMD by the serum endothelin-1 value. CMD = coronary microvascular dysfunction, AUC = area under the curve.

Figure 4. The distribution of CMD in patients with high endothelin-1 and patients with low endothelin-1. Blue represents CMD in patients without CMD in the non-obstructive territories. Red represents CMD in patients with CMD in the non-

obstructive territories. LM = left main artery, RCA = right coronary artery, ET-1 = endothelin-1, CFR_{remote} = coronary flow reserve in non-obstructive territories.

Table 1. Clinical characteristics of patients with 1- to 2-vessel disease or LM disease but non-obstructive RCA (n=49)

	CMD in non-obstructive territories (n=18)	**No CMD in non-obstructive territories (n=31)**	P-value
Age (yrs)	72 ± 8	70 ± 11	0.44
Male gender	15 (83%)	28 (90%)	0.47
Diabetes	11 (61%)	12 (39%)	0.13
Hypertension	13 (72%)	24 (77%)	0.68
Dyslipidemia	15 (83%)	24 (77%)	0.62
Smoking, current/ex-smoker	1 (6%) / 12 (67%)	3 (10%) / 19 (61%)	0.86
History of MI	6 (33%)	12 (39%)	0.71
History of PCI	6 (33%)	12 (39%)	0.71
History of CABG	3 (17%)	3 (10%)	0.47
ACE inhibitors	4 (22%)	6 (19%)	0.81
ARBs	7 (39%)	12 (39%)	0.99
Beta-blockers	13 (67%)	19 (61%)	0.71
Calcium blockers	6 (33%)	16 (52%)	0.22
Statins	15 (83%)	29 (94%)	0.26
Global rest MBF (mL/g/min)	0.98 ± 0.20	0.78 ± 0.22	0.003
Global stress MBF (mL/g/min)	1.53 ± 0.40	2.02 ± 0.52	0.001
Global CFR	1.57 ± 0.26	2.68 ± 0.61	<0.0001

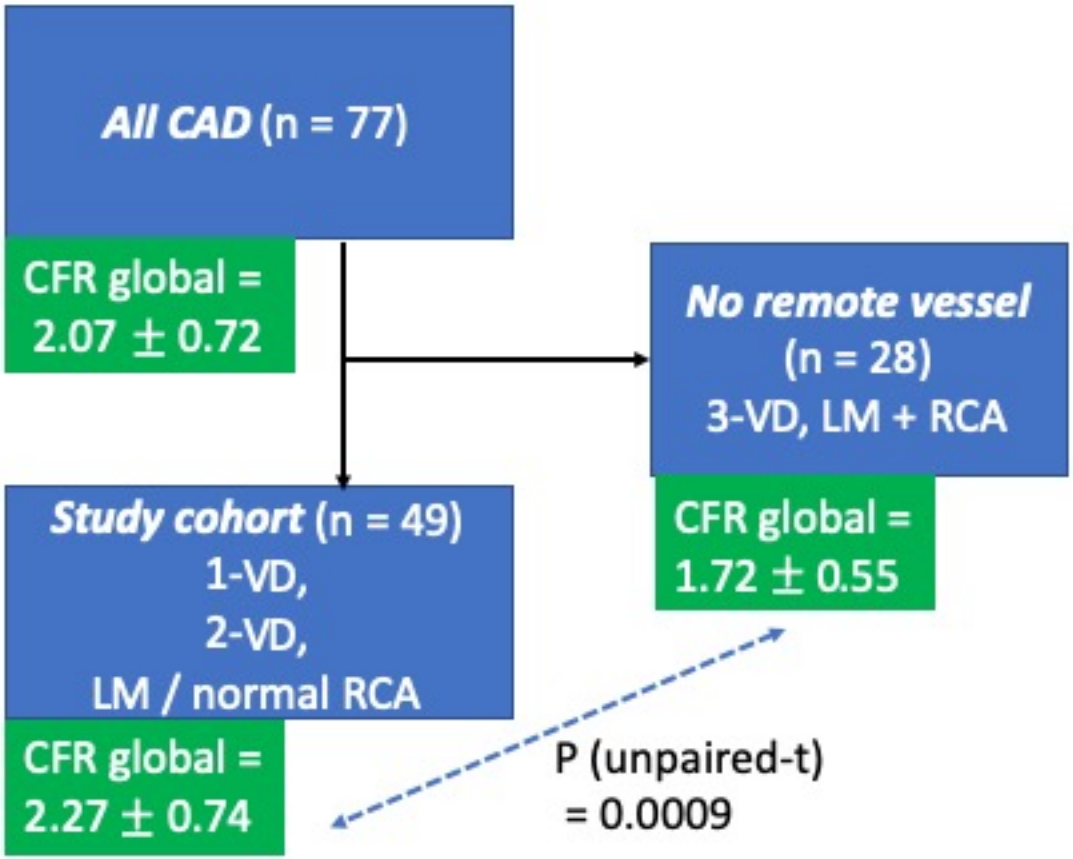
Continuous variables are presented as the median (interquartile range). Dichotomous variables are presented as n (%).

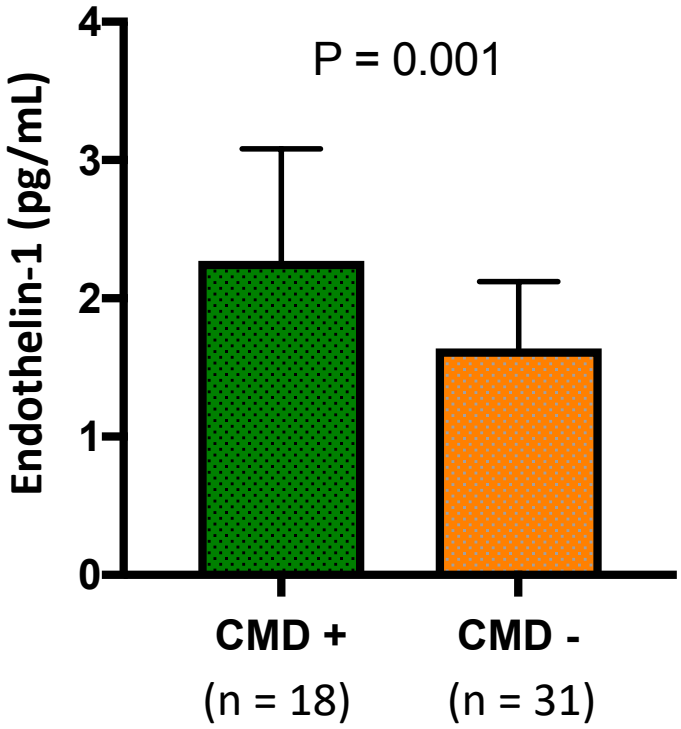
MI = myocardial infarction, PCI = percutaneous coronary intervention, CABG = coronary artery bypass grafting, ACE = angiotensin converting enzyme, ARB = angiotensin receptor blocker, MBF = myocardial blood flow, CFR = coronary flow reserve.

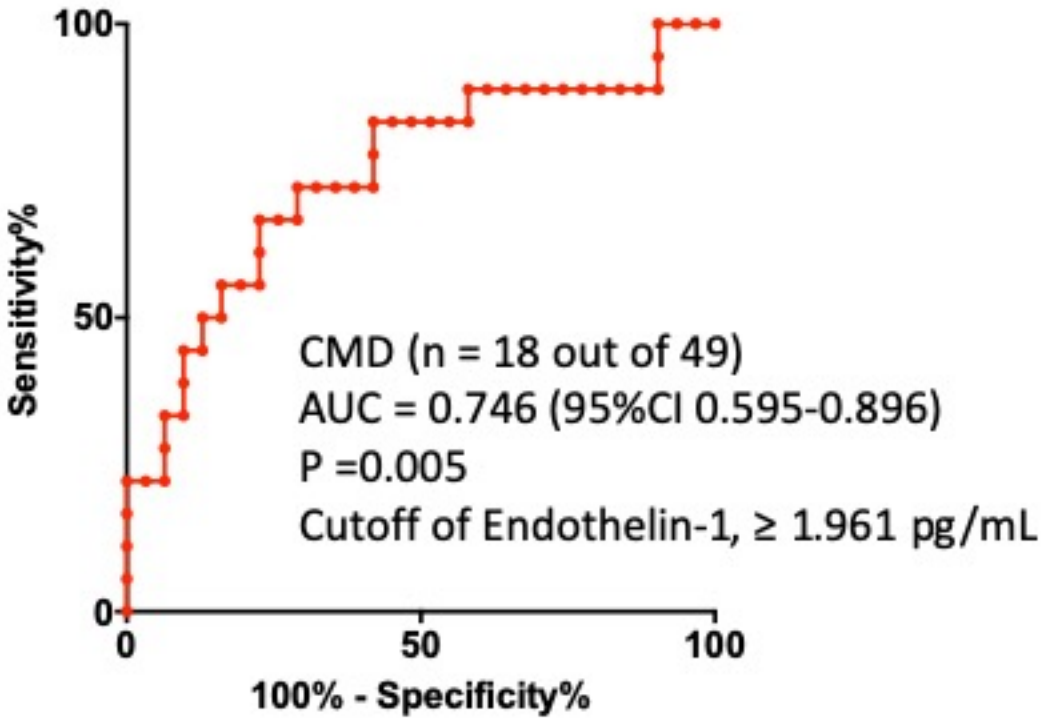
Table 2. Univariate and multivariate predictors of coronary microvascular dysfunction in non-obstructive territories

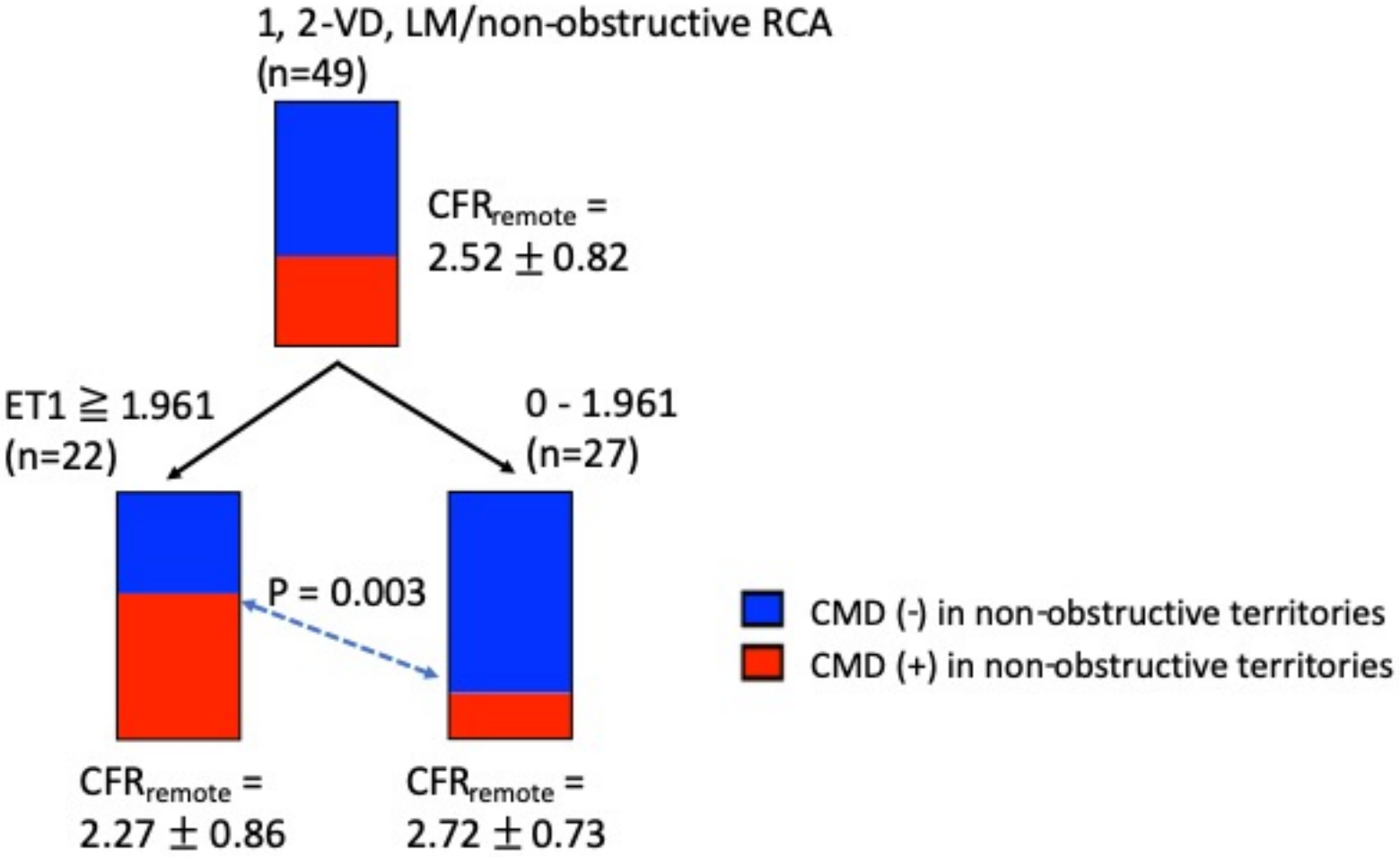
	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value
Age (y.o.)	1.03	0.97–1.09	0.43	---		
Male gender	0.54	0.10–2.99	0.48	---		
Hypertension	0.76	0.20–2.87	0.68	---		
Diabetes	2.49	0.76–8.19	0.13	2.70	0.72–10.15	0.14
Dyslipidemia	1.46	0.33–6.53	0.62	---		
Current smoker	0.55	0.05–5.71	0.62	---		
History of MI	0.79	0.23–2.68	0.71	---		
Endothelin-1 category (≥ 1.961 pg/mL)	6.36	1.75–23.10	0.005	6.64	1.75–25.23	0.005

CI = confidence interval, MI = myocardial infarction


All CAD (n = 77)
CFR global = 2.07 ± 0.72
No remote vessel (n = 28) 3-VD, LM + RCA
CFR global = 1.72 ± 0.55
Study cohort (n = 49) 1-VD, 2-VD, LM / normal RCA
CFR global = 2.27 ± 0.74
P (unpaired-t) = 0.0009


Endothelin-1 (pg/mL)
4
3
2
1
0
P = 0.001
CMD +
(n = 18)
CMD -
(n = 31)


100
50
0
Sensitivity%
0
50
100
100% - Specificity%
CMD (n = 18 out of 49)
AUC = 0.746 (95%CI 0.595-0.896)
P =0.005
Cutoff of Endothelin-1, ≥ 1.961 pg/mL


1, 2-VD, LM/non-obstructive RCA
(n=49)
CFR remote =
2.52 ± 0.82
ET1 ≧ 1.961
(n=22)
0 - 1.961
(n=27)
P = 0.003
CFR remote =
2.27 ± 0.86
CFR remote =
2.72 ± 0.73
CMD (-) in non-obstructive territories
CMD (+) in non-obstructive territories