



Plasma concentration of serotonin is a novel biomarker for coronary microvascular dysfunction in patients with suspected angina and unobstructive coronary arteries

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Aims

Although the importance of coronary microvascular dysfunction (CMD) has been emerging, reliable biomarkers for CMD remain to be developed. We examined the potential usefulness of plasma concentration of serotonin to diagnose CMD in patients with suspected angina and unobstructive coronary arteries.

Methods and results

We enrolled 198 consecutive patients (M/F 116/82, 60.2 ± 13.3 years old) who underwent acetylcholine provocation test and measured plasma serotonin concentration. Coronary microvascular dysfunction was defined as myocardial lactate production without or prior to the occurrence of epicardial coronary spasm during acetylcholine provocation test. Although no statistical difference in plasma concentration of serotonin [median (inter-quartile range) nmol/L] was noted between the vasospastic angina (VSA) and non-VSA groups [6.8 (3.8, 10.9) vs. 5.1 (3.7, 8.4), $P=0.135$], it was significantly higher in patients with CMD compared with those without it [7.7 (4.5, 14.2) vs. 5.6 (3.7, 9.3), $P=0.008$]. Among the four groups classified according to the presence or absence of VSA and CMD, serotonin concentration was highest in the VSA with CMD group. Importantly, there was a positive correlation between plasma serotonin concentration and baseline thrombolysis in myocardial infarction frame count ($P=0.001$), a marker of coronary vascular resistance. The classification and regression trees analysis showed that plasma serotonin concentration of 9.55 nmol/L was the first discriminator to stratify the risk for the presence of CMD. In multivariable analysis, serotonin concentration greater than the cut-off value had the largest odds ratio in the prediction of CMD [odds ratio (95% confidence interval) 2.63 (1.28–5.49), $P=0.009$].

Conclusions

Plasma concentration of serotonin may be a novel biomarker for CMD in patients with angina and unobstructive coronary arteries.

Keywords

Coronary artery spasm • Serotonin • Coronary microvascular dysfunction

Introduction

It has been reported that up to 40% of patients undergoing diagnostic coronary angiography for typical chest pain have no significant coronary stenosis.¹ The Women's Ischaemia Syndrome Evaluation study

showed that there are at least 3–4 million patients in the USA alone who have signs and symptoms of myocardial ischaemia despite no evidence of obstructive coronary artery disease (CAD), associated with poor quality of life, psychological distress, and health-care costs that approximate those of patients with obstructive CAD.^{2,3} In such

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cases, myocardial ischaemia may be caused by different types of functional disorders involving the epicardial coronary arteries, coronary microcirculation, or both.⁴ Vasospastic angina (VSA) is one of the important functional cardiac disorders characterized by myocardial ischaemia attributable to epicardial coronary artery spasm, and a number of studies have elucidated patient characteristics, outcomes, and prognostic factors of VSA.^{5–7} Especially, we have recently demonstrated that Rho-kinase activity in circulating neutrophils is enhanced in VSA patients and is a useful biomarker for diagnosis and disease activity assessment of the disorder.^{8,9} In contrast, coronary microvascular dysfunction (CMD) has emerged as a third potential mechanism of myocardial ischaemia in addition to coronary atherosclerotic disease and epicardial coronary spasm. Indeed, recent studies have demonstrated that CMD could be associated with increased risk of cardiovascular events.¹⁰ However, there is no reliable biomarker available for the diagnosis of CMD.

Serotonin, which is released from activated platelets, has been shown to cause both vasoconstriction and vasodilation by interacting with receptors on vascular smooth muscle cells and endothelial cells, respectively.¹¹ It has been shown that plasma concentration of serotonin was significantly higher in patients with CAD or VSA than in those without it.^{12,13} However, less attention has been paid to a possible relationship between serum concentration of serotonin and functional abnormalities of coronary microcirculation in patients with angina and no epicardial coronary stenosis. Thus, in this study, we examined whether plasma concentration of serotonin could be a novel biomarker for CMD in patient with suspected angina and unobstructive coronary arteries.

Methods

This study was conducted following the ethical principles in the Declaration of Helsinki. The protocol of this study was approved by the Ethics Committees of Tohoku University (No. 2011-417) and all patients provided written informed consent before study entry.

Study populations

From January 2011 to March 2014, a total of 1240 patients underwent elective diagnostic cardiac catheterization for evaluation of chest pain and/or electrocardiography abnormalities at our Tohoku University Hospital. Among them, 394 patients had no significant coronary stenosis (luminal narrowing <50%) of the major coronary arteries on control angiography and 252 underwent acetylcholine (ACh) provocation test.¹⁴ Patients with chronic renal failure on haemodialysis, known diagnosis of cardiomyopathies, history of out-of-hospital cardiac arrest, and unsuccessful evaluation for lactate level at coronary sinus during ACh provocation test were excluded. Finally, 198 consecutive patients, who fulfilled the inclusion criteria, were analysed in this study (Figure 1).

Acetylcholine provocation test and evaluation of coronary microvascular dysfunction

Acetylcholine provocation test was performed as reported previously,^{8,9} following the guidelines of the Japanese Circulation Society.¹⁴ Briefly, ACh was administered into the coronary artery in a cumulative manner (20, 50, and 100 µg) with careful monitoring of arterial pressure and 12-lead electrocardiography and serial coronary angiograms at 1-min intervals. Calcium channel blockers, long-acting nitrates, and nicorandil were

discontinued at least 48 h before the provocation test. To determine whether multivessel coronary spasm would develop, we first performed ACh provocation test for the left coronary artery (LCA) in a cumulative manner (20, 50, and 100 µg). If the test for the LCA was negative or ACh-induced spasms in the LCA resolved spontaneously, we then injected ACh into the right coronary artery in a cumulative manner (20 and 50 µg). When coronary spasm was induced, 5 mg of isosorbide dinitrate (ISDN) was injected into the responsible coronary artery.

To evaluate the presence of CMD, we estimated lactate production during myocardial ischaemia induced by ACh provocation test. Myocardial lactate extraction ratio was calculated as the ratio of the coronary arterial–venous difference in lactate concentration to the arterial concentration. Myocardial lactate production defined by negative myocardial lactate extraction ratio is considered to be high-sensitive to myocardial ischaemia.¹⁵ In this study, we defined CMD as myocardial lactate production despite the absence of angiographically demonstrable epicardial spasm throughout ACh provocation test or prior to the occurrence of epicardial coronary spasm following intracoronary injections of ACh.¹⁶ At 1 min after each dose of ACh was given to LCA, paired samples of 1 mL of blood were collected from the left coronary ostium and the coronary sinus for measurement of lactate concentrations, which was immediately determined with a calibrated automatic lactate analyser (ABL8000 FLEX system, RADIOMETER, Copenhagen, Denmark). We usually evaluate lactate production during ACh provocation test only in the LCA, as the great coronary sinus drains blood from the LCAs but not from the right coronary artery. Finally, we categorized the patients who were negative for both epicardial coronary spasm and myocardial lactate production during ACh provocation test as the chest pain syndrome group (Figure 1).

Measurement of plasma concentration of serotonin

Serotonin concentrations in platelet-poor plasma (PPP) and whole blood (WB) were measured using the high-performance liquid chromatography (HPLC) method with a column-switching and the post-column reaction as previously described.¹⁷ Briefly, blood samples were obtained from the left coronary ostium through a 5F catheter just before ACh provocation test and 2 mL of blood was dispensed into plastic vacuum tubes containing 3.6 mg/mL ethylenediamine tetraacetic acid (EDTA)-2K. The PPP samples were collected after centrifugation at 1000 g for 30 min at 4 °C. Serotonin was separated from the pre-treated samples by HPLC using a column switching system (HLC-725CAll; Tosoh Corp, Tokyo, Japan). Subsequently, serotonin was converted into a fluorescent derivative with benzylamine.¹⁵

Thrombolysis in myocardial infarction frame count

We previously demonstrated that the thrombolysis in myocardial infarction (TIMI) frame count, a marker of coronary blood flow, could reflect the presence of CMD.¹⁸ Using angiographic images recorded at 30 frames/s by biplane X-ray system, TIMI frame count was measured to first reach a standardized distal landmark in the left anterior descending (LAD) by two independent observers who were blinded to the assignment of patients.¹⁹

Statistical analysis

Continuous variables are expressed as mean ± standard deviation or median (inter-quartile range), and categorical variables as numeral (percentage). Welch's *t*-test for normal distribution and Wilcoxon rank sum test for asymmetric distribution [e.g. plasma serotonin, B-type natriuretic peptide, and high sensitivity C-reactive protein (hsCRP)] were used to analyse differences in continuous variables. Fisher's exact test was used for categorical variables. Relations between variables were determined

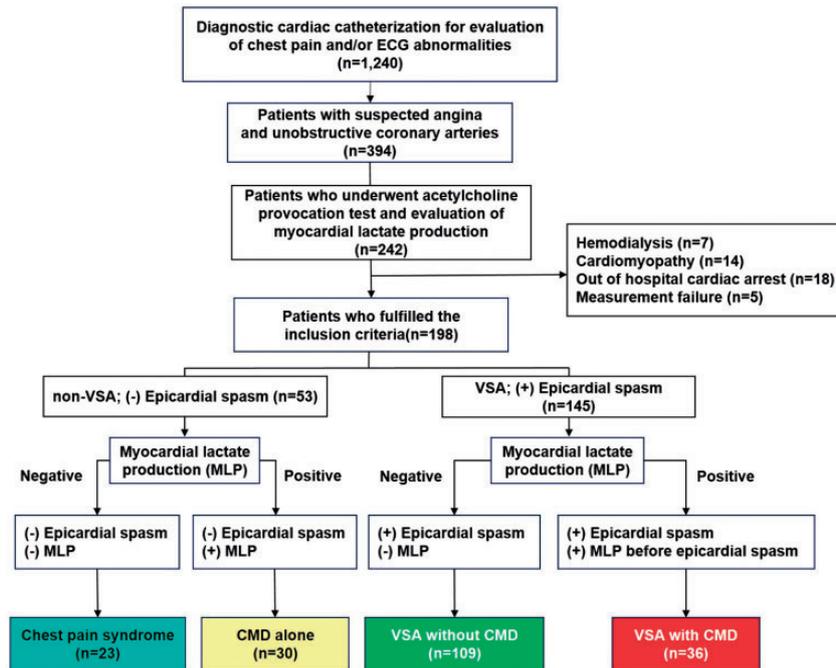


Figure 1 Study flowchart. VSA, vasospastic angina; CMD, coronary microvascular dysfunction; MLP, myocardial lactate production.

by Spearman coefficient analysis. Comparisons among groups were performed with Kruskal–Wallis test followed by Steel Dwass test. Classification and regression trees (CART) analysis, which is an empirical and statistical technique based on recursive partitioning of the data space to predict response,²⁰ was performed in order to identify the cut-off points of plasma serotonin concentration to diagnose the presence of CMD. The CART model was pruned so that the maximum depth of the final tree equal to two in order for a split to be attempted to avoid overfit. Logistic regression analysis was used to analyse the predictor for the presence of CMD by stepwise backward elimination with the following covariates; age, sex, left ventricular ejection fraction, haemoglobin, platelet count, chronic kidney disease, hsCRP, hypertension, dyslipidaemia, diabetes mellitus, smoking, and plasma concentration of serotonin. The statistical analysis was performed with SPSS statistics 22 (IBM Corp, Armonk, NY, USA) and R version 3.1.1 (<http://www.r-project.org>, 21 September 2016). A P -value <0.05 was considered to be statistically significant.

Results

Clinical characteristics of patients

Flow chart of this study is shown in *Figure 1*. In this study, we finally analysed 198 consecutive patients (M/F 116/82, 60.2 ± 13.3 years) who underwent ACh provocation test with simultaneous evaluation of plasma concentration of serotonin. They were divided into two groups, depending on their response of epicardial coronary arteries to the provocation test: the VSA group ($n = 145$) and non-VSA group ($n = 53$) (*Figure 1*). Patient characteristics were comparable between the VSA and the non-VSA groups (*Table 1*). When we defined CMD as myocardial ischaemia (lactate production) without, or prior to the

occurrence of epicardial coronary spasm during ACh provocation test, 66 patients (33.3%) had CMD. There was no difference in clinical characteristics between patients with CMD ($n = 66$) and those without it ($n = 132$), except for values of lactate extraction ratio (*Table 1*). The patients with CMD, as compared with those without it, had lower lactate extraction ratio at all points, including baseline, 1 min after maximum dose of ACh and after ISDN administration (*Table 1*). According to the presence or absence of epicardial coronary spasm and CMD, the subjects were further divided into the four groups, including chest pain syndrome ($n = 23$), CMD alone ($n = 30$), VSA without CMD ($n = 109$), and VSA with CMD ($n = 36$) groups (*Figure 1*). Clinical characteristics of the four groups are summarized in the Supplementary material online, *Table S1* Supplementary Table. In the CMD alone group, lactate extraction ratio decreased progressively even after ISDN was administered, suggesting that myocardial ischaemia was prolonged (see Supplementary material online, *Table S1*).

Plasma concentrations of serotonin by patient group classified according to acetylcholine provocation test

No statistical difference in plasma concentrations of serotonin was noted between the VSA group and non-VSA group [6.8 ($3.8, 10.9$) vs. 5.1 ($3.7, 8.4$) nmol/L, $P = 0.135$] (*Figure 2A*), whereas the concentrations were significantly higher in the CMD group than in the non-CMD group [7.7 ($4.5, 14.2$) vs. 5.6 ($3.7, 9.3$) nmol/L, $P = 0.008$] (*Figure 2B*). Among the four groups classified by the presence or absence of VSA and CMD, plasma concentration of serotonin was significantly higher in the VSA and CMD group than in the chest pain syndrome group [8.7 ($5.9, 14.0$) vs. 4.4 ($3.4, 5.8$) nmol/L, $P = 0.001$],

Table 1 Patient characteristics according to the results of acetylcholine provocation test

	Overall (n = 198)	Non-VSA (n= 53)	VSA (n= 145)	P-value	Non-CMD (n= 132)	CMD (n= 66)	P-value
Age (years)	60.2 ± 13.3	58.3 ± 16.8	60.9 ± 11.8	0.622	59.7 ± 13.2	61.2 ± 13.5	0.417
Male (%)	116(58.5)	28(52.8)	88(60.6)	0.333	82(62.1)	34(51.5)	0.170
Hypertension (%)	109(55.1)	30(56.6)	79(54.4)	0.872	70(53)	39(59)	0.451
Dyslipidaemia (%)	96(48.5)	23(43.3)	73(50.3)	0.424	65(49.2)	31(46.9)	0.880
Diabetes mellitus (%)	50(25.3)	11(20.7)	39(26.8)	0.461	32(24.2)	18(27.2)	0.729
Smoking history (%)	100(50.5)	21(39.6)	79(54.4)	0.078	69(52.2)	31(46.9)	0.650
LVEF (%)	70.7 ± 10.0	73.2 ± 7.8	69.9 ± 10.6	0.070	70.4 ± 11.2	71.5 ± 7.1	0.755
Haemoglobin (g/dL)	13.2 ± 1.8	13.2 ± 1.6	13.2 ± 1.9	0.829	13.3 ± 1.8	13.0 ± 1.7	0.341
Platelet (10 ³ /μL)	213.0 ± 58.9	207.1 ± 49.9	215.1 ± 61.9	0.360	212.2 ± 55.8	214.4 ± 65.0	0.852
eGFR (mL/min/1.73m ²)	73.1 ± 18.7	75.4 ± 21.6	72.3 ± 17.5	0.366	71.2 ± 17.4	76.9 ± 20.7	0.056
BNP (pg/mL)	20.2(8.7, 41.5)	25.8(10.8, 47.6)	18.8(8.5, 36.6)	0.130	19.5(8.7, 39.7)	20.9(9.8, 44.5)	0.577
hsCRP (mg/dL)	0.06(0.03, 0.1)	0.06(0.03, 0.12)	0.05(0.03, 0.1)	0.907	0.06(0.03, 0.1)	0.05(0.02, 0.1)	0.322
Lactate extraction ratio							
Baseline	0.2 ± 0.25	0.18 ± 0.25	0.21 ± 0.25	0.355	0.22 ± 0.26	0.16 ± 0.21	0.015
Maximum dose of ACh	-0.07±0.35	-0.01 ± 0.22	-0.08 ± 0.38	0.497	0.01 ± 0.33	-0.21 ± 0.35	<0.001
Post-ISDN administration	0.02 ± 0.28	-0.06 ± 0.35	0.05 ± 0.24	0.011	0.08 ± 0.24	-0.09 ± 0.31	<0.001

Results are expressed as mean ± standard deviation or n (%).

ACh, acetylcholine; BNP, B-type natriuretic peptide; CMD, coronary microvascular dysfunction; eGFR, estimate glomerular filtration rate; hsCRP, high sensitivity C-reactive protein; ISDN, isosorbide dinitrate; Lactate extraction ratio = (lactate in the aorta-lactate in the coronary sinus)/lactate in the aorta; LVEF, left ventricular ejection fraction; VSA, vasospastic angina.

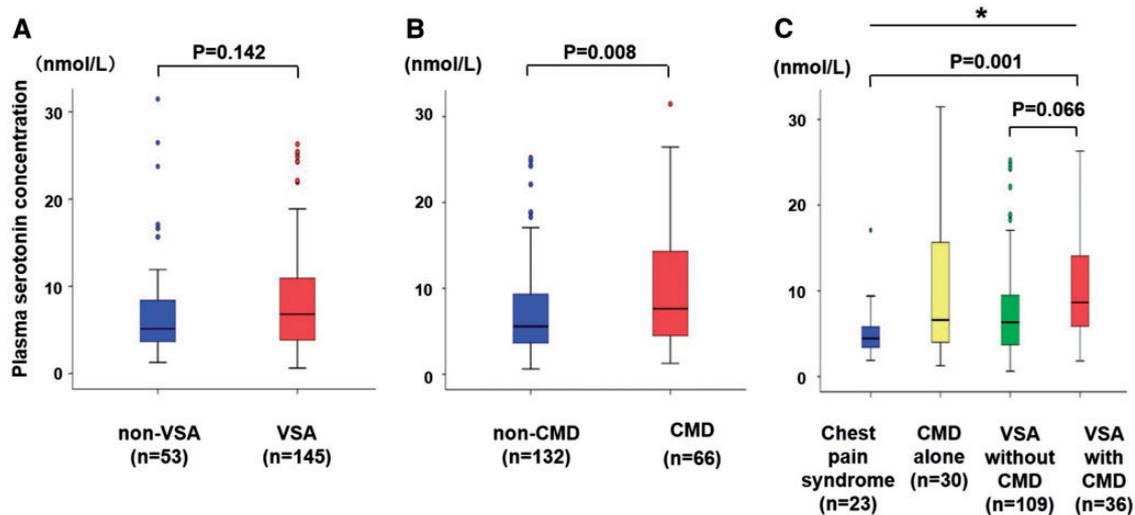


Figure 2 Plasma concentrations of serotonin by patient group classified according to the presence or absence of VSA and CMD. (A) Plasma concentrations of serotonin were compatible between the VSA and non-VSA groups. Results are expressed as box-and-whisker plots; the central box covers the interquartile range, with the median indicated by the line within the box. The whiskers extend to the most extreme values within 1.5 interquartile ranges. More extreme values are plotted individually. (B) Plasma concentrations of serotonin were higher in patients with CMD than in those without it. (C) Plasma serotonin concentrations of the four groups classified according to the presence or absence of VSA and CMD are shown. The serotonin concentrations were significantly higher in the VSA with CMD group than in the chest pain syndrome group by Steel–Dwass test. * $P < 0.01$ for the difference in plasma concentrations of serotonin among the four groups by Kruskal–Wallis test. VSA, vasospastic angina; CMD, coronary microvascular dysfunction.

and tended to be greater than in the VSA without CMD group [8.7 (5.9, 14.0) vs. 6.3 (3.7, 9.5) nmol/L, $P = 0.066$] (Figure 2C). In contrast, WB serotonin concentration was comparable among the four groups (see Supplementary material online, Supplementary Figure S1). Accordingly, as compared with the chest pain syndrome group, plasma/WB serotonin ratio, which is a marker of platelet activation,^{17,21} was significantly higher in the VSA with CMD group [1.3 (0.9, 2.2) vs. 0.8 (0.7, 1.1), $P = 0.04$] and the CMD alone group [1.8 (0.7, 3.3) vs. 0.8 (0.7, 1.1), $P = 0.019$] (see Supplementary material online, Figure S2 Supplementary Figure 2).

Correlation with thrombolysis in myocardial infarction frame count and plasma serotonin level

Thrombolysis in myocardial infarction frame count, a marker of coronary blood flow and vascular resistance, reflects the severity of CMD and thus is useful to diagnosis the disorder.¹⁸ We thus examined the correlation between TIMI frame count in LAD (TIMI FC-LAD) at baseline and plasma concentration of serotonin. As shown in Figure 3A, TIMI FC-LAD tended to increase in the patients with CMD irrespective of the presence or absence of epicardial coronary spasm. Importantly, there was a positive significant correlation between plasma concentration of serotonin and baseline TIMI FC-LAD ($r = 0.23$, $P = 0.001$) (Figure 3B).

Cut-off value of plasma concentration of serotonin to identify the presence of coronary microvascular dysfunction

The CART analysis was performed in order to identify the cut-off value of plasma serotonin concentration to classify the patients with

CMD. The analysis showed that the first discriminating point for the presence of CMD was plasma serotonin concentration of 9.55 nmol/L (Figure 4). Furthermore, multivariable logistic regression analysis showed that serotonin concentration greater than the cut-off value (>9.55 nmol/L) was the significant, independent, and most powerful predictor for the presence of CMD [odds ratio (95% confidence interval) 2.63 (1.28–5.49), $P = 0.009$] (Table 2). Hypertension has been reported as a risk factor for CMD.²² In this study, hypertension also remained in the final and best predictive model for CMD in patients with suspected angina and non-obstructive coronary arteries (Table 2).

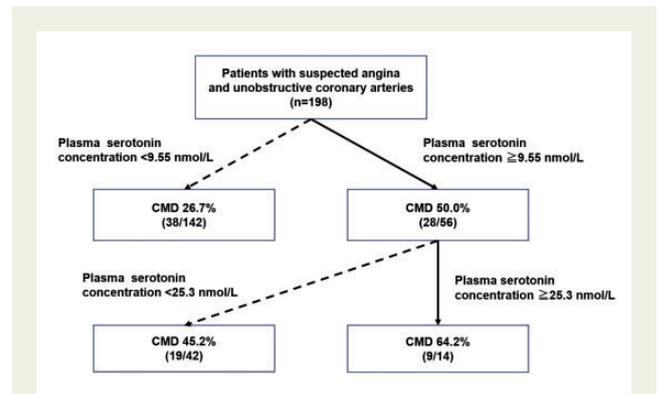


Figure 4 Classification and regression trees analysis for the presence of CMD. CMD, coronary microvascular dysfunction.

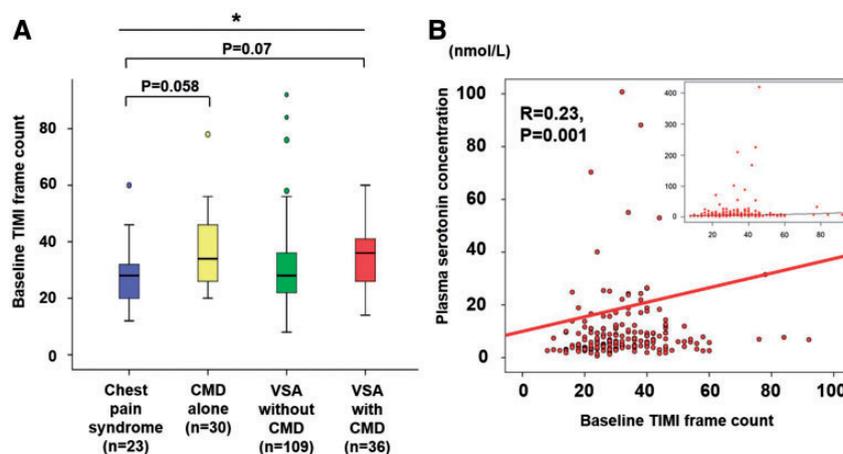


Figure 3 Thrombolysis in myocardial infarction (TIMI) frame count and plasma serotonin concentrations. (A) TIMI frame count in LAD (TIMI FC-LAD) of the four groups classified according to the presence or absence of VSA and CMD is shown. Results are expressed as box-and-whisker plots; the central box covers the interquartile range, with the median indicated by the line within the box. * $P < 0.01$ for the difference of TIMI FC-LAD of the four groups classified according to the presence or absence of VSA and CMD by Kruskal–Wallis test. (B) Relationship between plasma concentrations of serotonin and TIMI FC-LAD. VSA, vasospastic angina; CMD, coronary microvascular dysfunction.

Table 2 Logistic regression analysis for the presence of coronary microvascular dysfunction in patients with suspected angina and unobstructive coronary arteries

	Univariate analysis					Stepwise analysis				
	Coefficient	Standardized coefficient	OR	95% CI	P-value	Coefficient	Standardized coefficient	OR	95% CI	P-value
Age	0.009	0.012	1.009	0.986–1.032	0.451					
Sex	−0.434	0.305	0.648	0.357–1.177	0.153					
LVEF	0.011	0.016	1.011	0.98–1.044	0.484					
Haemoglobin	−0.080	0.085	0.923	0.782–1.091	0.348					
Platelet	0.001	0.003	1.001	0.996–1.006	0.811					
CKD	−0.183	0.363	0.833	0.409–1.698	0.615					
BNP	−0.003	0.003	0.997	0.991–1.003	0.326					
hsCRP	0.473	0.786	1.604	0.344–7.481	0.548					
Hypertension	0.246	0.305	1.279	0.704–2.327	0.419	0.573	0.366	1.774	0.875–3.703	0.118
Dyslipidaemia	−0.091	0.302	0.913	0.505–1.65	0.763					
Diabetes mellitus	0.159	0.343	1.172	0.598–2.295	0.644					
Smoking history	−0.153	0.305	0.858	0.472–1.559	0.614					
Plasma serotonin concentration > 9.55 nmol/L	1.007	0.328	2.737	1.44–5.202	0.002	1.031	0.383	2.803	1.330–6.004	0.007

The ORs are for a 1 unit difference.

BNP, B-type natriuretic peptide; CI, confidence interval; CKD, chronic kidney disease; CMD, coronary microvascular dysfunction; hsCRP, high sensitivity C-reactive protein; LVEF, left ventricular ejection fraction; OR, odds ratio.

Discussion

The major findings of this study were that (i) plasma concentration of serotonin was significantly higher in patients with CMD, (ii) there was a positive correlation between plasma concentration of serotonin and TIMI FC-LAD at baseline, and (iii) plasma concentration of serotonin above the cut-off value (9.55 nmol/L) was the strongest predictor for diagnosis of CMD. To the best of our knowledge, this is the first study that demonstrates the clinical significance of plasma concentration of serotonin as a useful biomarker for CMD in patients with angina and unobstructive coronary arteries.

Plasma concentration of serotonin in patients with suspected angina and unobstructive coronary arteries

Serotonin is released from aggregating platelets, causing vasoconstriction, and platelet aggregation with cyclic flow reduction.²³ Moreover, it also acts as a growth factor that stimulates proliferation and migration of vascular smooth muscle cells and promotes atherosclerosis.²⁴ Serum concentration of serotonin is increased in stenosed canine coronary arteries²⁵ and reflects the extent of arteriosclerotic changes and subsequent cardiac events in humans.¹² Several clinical studies previously addressed the relationship between systemic serotonin concentrations and coronary vasomotor dysfunction with a small number of patients with inconsistent results.^{13,21,26} This study demonstrates that plasma concentration of serotonin is not affected by the presence or absence of epicardial coronary spasm, whereas the concentrations were significantly elevated in patients with CMD. Furthermore, the serotonin concentrations

were significantly elevated in the VSA with CMD group as compared with the chest pain syndrome group. Plasma to WVB serotonin ratio, a sensitive biomarker of platelet activation,¹⁷ was significantly higher in the VSA with CMD and the CMD alone groups than in the chest pain syndrome group. In contrast, plasma concentrations of serotonin were comparable between the VSA without CMD group and the chest pain syndrome group. These results suggest that plasma concentrations of serotonin reflect abnormal reactivity of coronary resistance vessels rather than that of epicardial coronary arteries. Indeed, in this study, there was a positive correlation between plasma serotonin concentration and baseline TIMI FC-LAD, a marker of coronary vascular resistance for CMD.¹⁸ Thus, the present finding suggests that plasma concentration of serotonin is a useful biomarker to dissect CMD from epicardial coronary artery spasm.

Significance of increased plasma concentration of serotonin in patients with coronary microvascular dysfunction

Coronary microvascular dysfunction is caused by a variable combination of impaired coronary microvascular dilatation, both endothelium-dependent and -independent mechanisms, and enhanced coronary microvascular constriction.¹⁰ Serotonin is well known to act as a potent bidirectional vasoactive substance, causing vasodilation via 5-HT₁ receptors on endothelial cells and vasoconstriction via 5-HT₂ receptors on vascular smooth muscle cells.^{11,27} Furthermore, serotonin could cause selective intense microvascular constriction with minimal effects on epicardial coronary arteries.²⁸ Indeed, it has been proposed that enhanced constriction of coronary

resistance vessels is the most important microvascular alteration in patients with CMD.²⁹ Enhanced vasoconstriction of coronary microvessels can be caused by either increased release of vasoconstrictor agonists or increased susceptibility of vascular smooth muscle cells to vasoconstrictor stimuli. In this study, the increased serotonin levels in patients with CMD could enhance coronary microvascular tone at baseline, as reflected by increased TIMI FC-LAD, through direct vasoconstrictor effect via 5-HT₂ receptors and sensitization of coronary microvessels to other vasoconstrictor agents, such as endothelin-1, catecholamines, and angiotensin II, all of which may play an important role in the pathogenesis of CMD.^{30,31}

The causes of CMD may be heterogeneous.⁹ Classical coronary risk factors are associated with impaired coronary microvascular dilatation and enhanced coronary microvascular constriction.^{10,32} Recently, low-grade inflammation attracts much attention in the pathogenesis of CMD, as CRP levels correlate with the frequency of angina attacks and impairment of coronary microvascular dilatation in patients with syndrome X.³³ However, in this study, hsCRP levels were comparable between patients with CMD and those without CMD, whereas plasma concentrations of serotonin were significantly elevated in CMD patients, suggesting that plasma concentrations of serotonin are more closely related to CMD as compared with low-grade inflammation. Additionally, the results of univariate and multivariate logistic regression analysis indicate that elevated serotonin concentrations are the most highly correlated with CMD in patients with angina and unobstructive CAD.

Clinical implications of measurement of plasma concentration of serotonin

Patients with stable angina in the absence of obstructive CAD have 1.5-fold higher mortality rate compared with healthy subject and ~40% of them experience repeat coronary angiography or re-hospitalization for chest pain.³⁴ Particularly, in patients with CMD, symptoms often persist despite full classical anti-ischaemic medications including calcium channel blockers and nitrates, both of which are effective in VSA patients.³⁵ Instead, angiotensin-converting enzyme inhibitors, statins, and newer antianginal drugs, such as ranolazine and ivabradine, improve symptoms in CMD patients.³⁵ Moreover, it has been recently reported that CMD is a predictor of a worse outcome.³⁶ Thus, identification of patients with CMD is important for appropriate management of those patients. As demonstrated in this study, plasma concentration of serotonin may be a useful biomarker for CMD in patients with angina-like chest pain and unobstructive coronary arteries. As coronary organic stenosis is also likely to cause elevation of serotonin concentrations,^{12,25} we should pay particular attention to the fact that the benefit of plasma concentration of serotonin as a biomarker for CMD is confined to angina patients with unobstructive coronary arteries. The advantages of this method are that it is non-invasive and requires only a small volume of blood, and thus can be easily performed in the clinical setting. Additionally, invasive provocative testing could be omitted if serum serotonin concentration was highly indicative of the presence of CMD. On the other hand, serotonin is not only a possible diagnostic tool for CMD but also a novel therapeutic target in patients with CMD. As discussed above, increased circulating concentrations of serotonin in patients with CMD could increase coronary

microvascular tone at baseline and enhance susceptibility of vascular smooth muscle cells to vasoconstrictor stimuli. A serotonin blocker, sarpogrelate, which has been reported to improve exercise capacity in patients with stable effort angina, has the potential to restore microvascular malfunction.³⁷ Further studies are needed to elucidate the roles of serotonin in the pathogenesis of CMD.

Study limitations

Several limitations should be mentioned for this study. First, this study was a single-centre and exploratory study. Future validation study regarding the present findings is needed. Second, we did not evaluate coronary flow reserve to document impaired coronary microvascular dilatation, which would involve endothelium-dependent and -independent mechanisms with a Doppler-tipped guidewire or other imaging modalities. Thus, it is conceivable that CMD was underdiagnosed in this study. Third, we did not perform ACh provocation test for RCA in all patients. As anatomically the great coronary sinus drains blood from the left coronary system but not from the right coronary system, we were unable to measure myocardial lactate production for RCA during ACh provocation test. Thus, functional evaluation for RCA may be incomplete in this study. Fourth, although CMD may be caused by various pathophysiologic mechanisms, the results of this study may only be applicable to patients with ACh-induced CMD (i.e. microvascular spasm). Thus, it remains to be elucidated whether the findings of this study could be extrapolated to patients with CMD due to other causes.

Conclusions

This study demonstrates that plasma serotonin concentration is a useful and novel biomarker for CMD in patients with angina and unobstructive coronary arteries.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: H.S. has received lecture fees from Bayer Yakuhin, Ltd (Osaka, Japan) and Daiichi Sankyo Co., Ltd (Tokyo, Japan).

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