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Measurement of myocardial lactate production for diagnosis of coronary microvascular spasm

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Corresponding Author:	Hiroaki Shimokawa Tohoku University School of Medicine: Tohoku Daigaku Daigakuin Igakukei Kenkyuka Igakubu Sendai, JAPAN
Corresponding Author's Institution:	Tohoku University School of Medicine: Tohoku Daigaku Daigakuin Igakukei Kenkyuka Igakubu
Corresponding Author E-Mail:	shimo@cardio.med.tohoku.ac.jp
Order of Authors:	Jun Takahashi
	Akira Suda
	Yasuda Satoshi
	Hiroaki Shimokawa
Additional Information:	
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TITLE:

Measurement of Myocardial Lactate Production for Diagnosis of Coronary Microvascular Spasm

AUTHORS AND AFFILIATIONS:

Jun Takahashi[1], Akira Suda[1], Satoshi Yasuda[1], Hiroaki Shimokawa[1]*

[1]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Email addresses of the authors:

Jun Takahashi (jtakahashi@cardio.med.tohoku.ac.jp)
Akira Suda (akira5885@cardio.med.tohoku.ac.jp)
Satoshi Yasuda (syasuda@cardio.med.tohoku.ac.jp)
Hiroaki Shimokawa (shimo@cardio.med.tohoku.ac.jp)

*Email address of the corresponding author:

Hiroaki Shimokawa (shimo@cardio.med.tohoku.ac.jp)

KEYWORDS:

lactate, coronary sinus, myocardial ischemia, coronary microvascular spasm, acetylcholine provocation testing, angina, INOCA

SUMMARY:

Myocardial lactate production (coronary arterial-venous difference in serum lactate level) during coronary spasm provocation testing is considered as a highly sensitive marker that reflects acetylcholine-induced myocardial ischemia due to microvascular spasm. This article presents the procedures to assess myocardial lactate production for the diagnosis of coronary microvascular spasm.

ABSTRACT:

In about a quarter of patients with angina and non-obstructive coronary arteries, no epicardial spasm is noted on coronary arteriography during an angina attack. Since the pressure-rate product is almost identical at rest and the onset of attack in those patients, the decrease in coronary blood flow rather than increased myocardial oxygen consumption is likely to explain myocardial ischemia, indicating a substantial involvement with coronary microvascular spasm (MVS). Myocardial lactate production, which could be defined as a negative myocardial lactate extraction ratio (ratio of the coronary arterial-venous difference in lactate concentration to arterial concentration), is considered indicative of objective evidence to support the emerging myocardial ischemia. Thus, monitoring of the myocardial lactate production and the emergence of chest pain and ischemic electrocardiographic changes during acetylcholine (ACh) provocation testing is of significant value for detecting the entity of MVS. Practically, 1 min after incremental doses of ACh (20, 50, and 100 μg) are administered into the left coronary artery (LCA), paired samples of 1 mL of blood are collected from the LCA ostium and coronary sinus for measurement of lactate concentration by a calibrated automatic lactate analyzer. Then, the development of

MVS could be confirmed by negative myocardial lactate extraction ratio despite the absence of angiographically demonstrable epicardial coronary spasm or before its occurrence throughout ACh provocation testing. In conclusion, assessment of myocardial lactate production is essential and valuable for the diagnosis of MVS.

INTRODUCTION:

Recent studies demonstrated that ischemia with non-obstructive coronary arteries (INOCA) is caused mainly by functional coronary vasomotion disorders, including epicardial and microvascular spasms[1]. The diagnosis of coronary vasoconstrictor dysfunction at the epicardial and/or microvascular levels often requires intracoronary provocation testing with a pharmacological vasoactive agent such as acetylcholine (ACh) during coronary angiography[2]. Many patients with INOCA have no epicardial spasm on coronary arteriography despite the development of angina attack and ischemic electrocardiographic (ECG) changes in response to intracoronary ACh[3]. Since the pressure-rate product is almost identical at rest and the onset of attack in those patients, the decrease in coronary blood flow rather than increased myocardial oxygen consumption is likely to explain myocardial ischemia, indicating a substantial involvement with coronary microvascular spasm (MVS). Additionally, MVS also seems to be involved in angina in a quarter of patients with vasospastic angina (VSA) due to epicardial coronary spasm[4].

Since no technique is available for visualizing coronary microvessels in humans *in vivo*, MVS is defined as ischemic ECG changes associated with the reproduction of usual chest pain in the absence of epicardial spasm (90%) intracoronary provocation testing[5]. Usually, upon the development of ischemia, myocardial lactate consumption decreases, and a shift to lactate production occurs as myocardial ischemia increases in severity[6,7]. Thus, an additional myocardial lactate production measurement is considered to be useful in confirming ACh-induced microvascular myocardial ischemia during provocation testing[3,4,8]. Here, the current protocol presents coronary sinus (CS) lactate measurements for the diagnosis of MVS.

PROTOCOL:

The measurement of myocardial lactate production during ACh provocation testing to evaluate coronary vasoreactivity was conducted following the ethical principles in the Declaration of Helsinki, and the protocol was approved by the Ethics Committees of Tohoku University (No.2016-1-643). All the patients provided written informed consent before the procedure. In this article, ACh provocation testing was performed following the guidelines of the Japanese Circulation Society[9].

1. Preparation for the procedure

1.1. Ensure that the measurement of myocardial lactate production is performed in patients undergoing ACh provocation testing to diagnose VSA and/or microvascular angina (MVA) due to vasospasm.

1.2. Ensure that the patients discontinue all vasoactive agents for accuracy of those diagnoses, including calcium channel blockers, long-acting nitrates, and nicorandil, at least 48 h before

catheterization study[9].

1.3. Do shave hair at puncture sites, including both inguinal regions and wrists.

2. Insertion of catheters before ACh provocation testing

2.1. Use local anesthesia at puncture sites with subcutaneous 1% lidocaine to insert intravenous and radial artery sheaths.

NOTE: The anesthesia effect is confirmed by the loss of pain sensation at the anesthetized area by pricking with a needle.

2.2. Place two 5 Fr venous sheaths through the right or left femoral vein with ultrasound guidance.

NOTE: One venous sheath is used to insert a temporary pacing electrode in the right ventricle in case of severe bradycardia after intracoronary ACh. The other one is for a CS catheter to obtain blood samples to measure lactate levels in the CS.

2.3. Place a 5 or 6 Fr arterial sheath through the radial or femoral artery.

2.4. Administer intravenous heparin (50 to 70 U/kg) to achieve therapeutic anticoagulation (activated clotting time ~250 s) before coronary instrumentation.

2.5. Canulate a 5 Fr or 6 Fr Judkins-left catheter into LCA through the radial or femoral artery.

NOTE: The usual catheter manipulations are performed with the Judkins left catheter.

2.6. Advance a CS catheter, for often a hydrophilic coating Amplatz-left catheter is used, from a venous sheath placed at the right femoral vein to the right atrium.

2.7. Confirm the configuration of CS and the location of its orifice in the right atrium in advance by detecting the CS image in the venous phase of LCA angiography (**Figure 1A**).

2.8. Canulate an Amplatz-left catheter into CS by turning the catheter counterclockwise at the right atrium with the left anterior oblique (LAO) view.

2.9. Verify whether the catheter is cannulated into CS and its position in CS is adequate by contrast injection from the end of the catheter (**Figure 1B**).

NOTE: The venous phase of the LCA angiography confirms whether the catheter is cannulated into CS.

2.10. Take a pair of blood samples from the CS and the ostium of LCA simultaneously to

examine myocardial lactate metabolism at baseline. Then, measure lactate levels in those samples using blood gas analysis equipped with automatic lactate measurement function.

3. Measurement of myocardial lactate production during ACh provocation testing

3.1. Perform the baseline left coronary angiography in an appropriate projection that ensures the best separation of the branches of each coronary artery, and serial angiographies after intracoronary injection of ACh should be performed in the same projection.

NOTE: Since the great coronary sinus drains blood from perfusion regions of the LCA but not from the right coronary artery, evaluation of myocardial lactate production is possible only for the LCA during ACh provocation testing[8,10].

3.2. Administer ACh into the coronary artery in a cumulative manner (ACh 20, 50, and 100 µg in 10 mL of solution) over 20 s with careful monitoring of blood pressure and 12-lead electrocardiography (ECG). Perform coronary angiography when chest pain or any ECG ST-segment change occurs, or routinely after completing each ACh injection[9,11].

3.3. Collect paired samples of 1 mL of blood from the LCA ostium and the CS to measure lactate concentrations at 1 min after each dose of ACh is given to LCA and determine lactate concentrations with a calibrated automatic lactate analyzer.

3.4. Calculate the lactate extraction ratio (LER) by dividing the coronary arteriovenous difference in the lactate concentration by the arterial lactate concentration as follows[4,8,10]:

LER = (arterial lactate concentration [mmol/L] – coronary venous lactate concentration [mmol/L])/arterial lactate concentration (mmol/L).

NOTE: Myocardial lactate production defined by negative LER is objective evidence to support the emerging myocardial ischemia[4,8,10]. Therefore, the occurrence of MVS as myocardial lactate production (negative LFR) becomes recognizable without or before the occurrence of angiographically apparent epicardial coronary spasm during ACh provocation testing[3].

3.5. Administer 5 mg of isosorbide dinitrate into the LCA if epicardial coronary spasms were induced. Promptly, perform coronary angiography while the coronary artery is maximally dilated.

3.5.1. Simultaneously, collect blood samples of 1 mL of blood from the LCA ostium and the CS to measure lactate concentrations after the relief of ACh-induced spasm.

REPRESENTATIVE RESULTS:

A 56-year-old woman with no coronary risk factors suffered from transient chest discomfort at rest. She underwent ACh provocation testing and measurement of myocardial lactate production for diagnosis of MVS. As shown in **Figure 2**, chest pain, ischemic ECG changes, and negative LER were noted immediately following 100 µg of ACh administration into the LCA. Still, no relevant

epicardial coronary spasm was observed on angiography. Thus, she was diagnosed as having MVS. Intriguingly, she had persistent negative LER even after isosorbide dinitrate (ISDN) was administered into the LCA, suggesting that myocardial ischemia attributable to impaired bioavailability of nitric oxide in coronary pre-arterioles was prolonged.

FIGURE AND TABLE LEGENDS:

Figure 1: A blood sampling setting for measuring lactate concentrations in LCA and CS (LAO 50°). A Judkins-left catheter was introduced into LCA (black arrow). To detect the CS orifice and visualize its whole configuration, the venous phase of LCA angiography (white arrows) is applicable (**A**). Regarding the CS imaging obtained at the venous phase of LCA angiography, an Amplatz-left catheter (white outlined arrow) was inserted through the right femoral vascular access into CS (white arrows) reliably and safely (**B**). CS indicates coronary sinus; LAO is left anterior oblique; LCA is left coronary artery.

Figure 2: Coronary angiograms, ECG changes, and lactate levels during ACh provocation testing in a 56-year-old female patient with repetitive resting angina attacks. Baseline coronary angiogram of LCA and ECG findings were normal (**A**). Intracoronary 100 μg of ACh induced reproduction of her usual symptoms and marked ST-segment depression in V_2-V_4 (red arrows), but no epicardial coronary vasoconstriction was noted (**B**). Changes in myocardial lactate metabolism throughout ACh provocation testing are summarized (**C**). LER, which is calculated as the ratio of the coronary arteriovenous difference in lactate concentration to arterial concentration, turned negative just after administration of 100 μg of acetylcholine, reflecting myocardial ischemia. ACh indicates acetylcholine; CS is coronary sinus; ISDN is isosorbide dinitrate; LCA is left coronary artery; LER is lactate extraction ratio.

DISCUSSION:

Detection of enhanced coronary vasoconstriction is possible by an additional pharmacological provocation testing with ACh or ergometrine during coronary angiography. Even now, there is no technique to directly visualize the coronary microvasculature for evaluation of its function *in vivo*, the occurrence of coronary spasm at microvascular level could be solely deduced by the reproduction of usual symptoms together with ischemic ECG changes despite the absence of epicardial coronary spasm during ACh provocation testing. Notably, an additional measurement of myocardial lactate production, a highly sensitive surrogate marker for myocardial ischemia, confirms the presence of myocardial ischemia objectively throughout provocative testing. Mohri et al. demonstrated that myocardial lactate production was noted during intracoronary ACh-induced angina attack in 9 of the 11 patients (82%) without epicardial coronary spasm. However, it was observed in none of 10 patients with atypical chest pain who showed a comparable degree of epicardial constriction induced by ACh[3]. Furthermore, about 25% of patients with vasospastic angina (VSA) caused by epicardial coronary spasm could be associated with MVS[4]. They are prevalent in women and often have prolonged and drug-tolerant seizures[4]. Since emerging MVS could be detected by myocardial lactate production before the occurrence of angiographic epicardial spasm[8], high-risk VSA patients can be dissected with both microvascular and epicardial spasms from those with epicardial spasm alone.

Measurement of myocardial lactate production during ACh provocation testing is safe and straightforward from a technical point of view. Indeed, the procedure's success depends on the cannulation into CS. Therefore, as shown in **Figure 1**, it is crucial to identify the location of CS orifice utilizing the venous phase imaging of LCA angiography before attempting to insert a catheter into CS. This process contributes to the ease of cannulation into CS and prevents complications, including CS dissection, perforation of CS or right atrium, and resultant cardiac tamponade. Actually, in the previous study with 198 patients who underwent the evaluation of myocardial lactate production during ACh provocation testing, no complication associated with the cannulation into CS was noted[8].

Occasionally, however, there is a failure to insert a catheter to collect blood samples into CS. The anatomic location of CS ostium to the right atrium is the crucial point for successful cannulation. When an Amplatz-left type catheter is advanced from the right femoral vein, catheter insertion into CS is often tricky in cases with CS ostium too close to or too far from the opening of the inferior vena cava. In such cases, vascular access for the CS catheter must be changed from the femoral vein to the internal jugular vein to complete cannulation into CS. In contrast, the procedure under systemic heparinization entails a risk of bleeding complications. Thus, the change of puncture site for the CS catheter should be determined in terms of clinical risk and benefit of evaluation of myocardial lactate production during ACh provocative testing.

In conclusion, measurement of myocardial lactate production is essential and valuable for the diagnosis of MVS, and the procedure is generally safe and straightforward, although it requires some experience.

ACKNOWLEDGMENTS:
We thank all the staff of the catheterization laboratory of the Tohoku University Hospital.

DISCLOSURES:
H.S. received lecture fees from Bayer Yakuhin, Ltd. and Daiichi Sankyo Co. Ltd., but declares no conflicts of interest regarding the present work. All the other authors have nothing to disclose.

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Figure 1

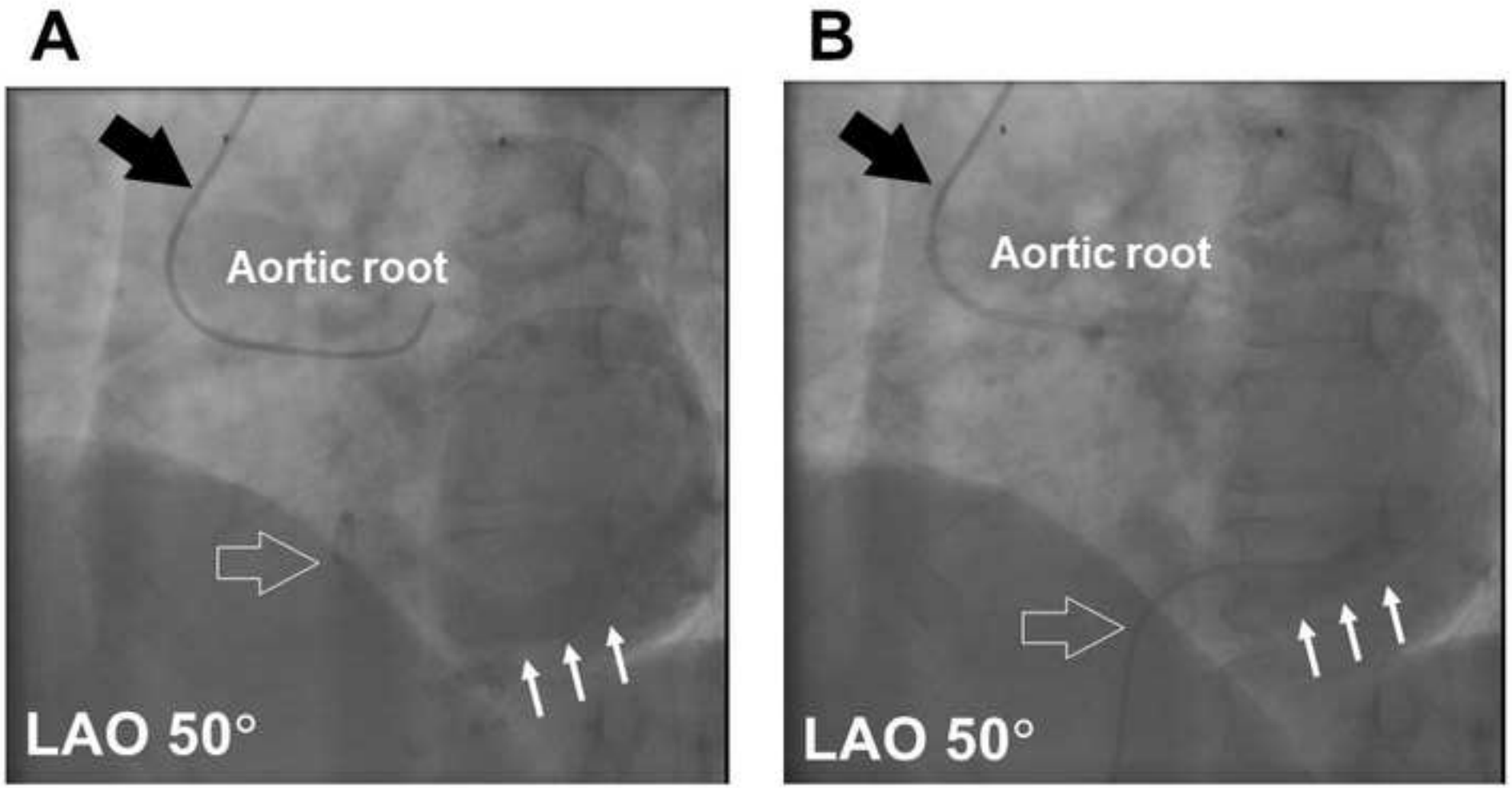
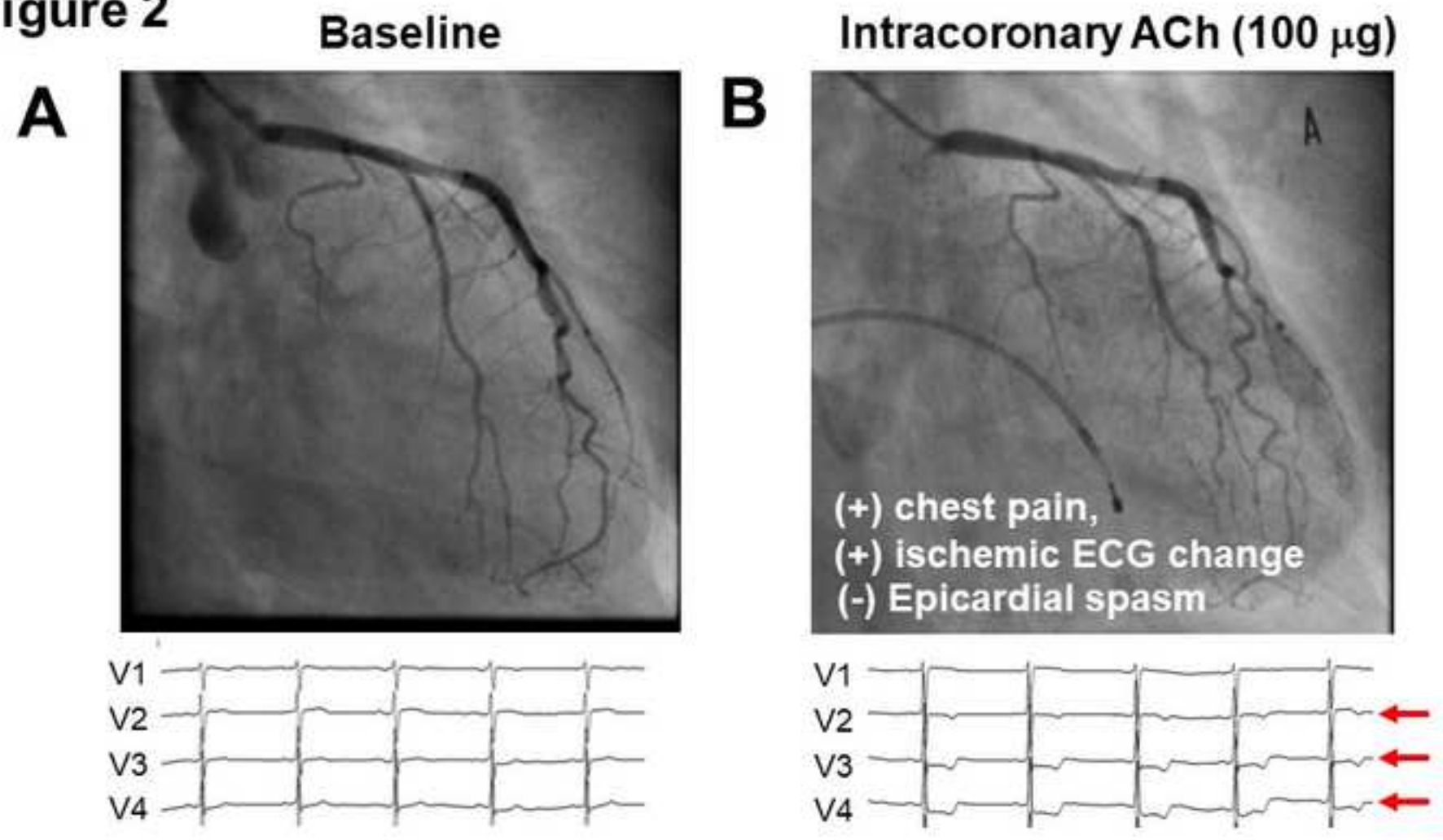


Figure 2



C

	Baseline	ACh 20 μg	ACh 50 μg	ACh 100 μg	After ISDN
Lactate levels (mmol/L) LCA	0.4	0.3	0.3	0.3	0.3
CS	0.3	0.2	0.3	0.4	0.4
LER	0.25	0.33	0	- 0.33	- 0.33

Responses to the Editor

MS.: JoVE62558/R1
Title: Measurement of myocardial lactate production for diagnosis of coronary microvascular spasm.

We would like to thank the Editors for the valuable comments. In line with the comments, we have revised our manuscript. In order to facilitate the review process, our point-to-point responses are shown in red font.

Responses to Editorial comments;

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

(Response)

Thank you very much for this valuable suggestion. In line with the Editor's comment, we have proofread our manuscript thoroughly and revised all spelling and/or grammatical errors.

2. Please revise the following lines to avoid previously published work: 37-40, 45-48, 56-58, 69-71, 74-76, 80-82, 139-141, 164-166, 212-214.

(Response)

Thank you very much for this valuable suggestion. In line with the Editor's comment, we have revised the descriptions that overlapped with the previously published work.

(Summary section) (Page 1, lines 37-41.)

Myocardial lactate production (coronary arterial-venous difference in serum lactate level) during coronary spasm provocation testing is considered as a highly sensitive

marker which reflects acetylcholine-induced myocardial ischemia due to microvascular spasm. In this article, we present our procedures to assess myocardial lactate production for diagnosing coronary microvascular spasm.

(Abstract section) (Page 2, lines 46-50.)

In about a quarter of patients with angina and non-obstructive coronary arteries, no epicardial spasm is noted on coronary arteriography during angina attack. Since pressure-rate product is almost identical at rest and at the onset of attack in those patients, the decrease in coronary blood flow rather than increased myocardial oxygen consumption is a likely explanation for myocardial ischemia, indicating a substantial involvement with coronary microvascular spasm (MVS).

(Abstract section) (Page 2, lines 56-60.)

Practically, one minute after incremental doses of ACh (20, 50, and 100 μg) are administered into the left coronary artery (LCA), paired samples of 1 mL of blood are collected from the LCA ostium and coronary sinus for measurement of lactate concentration by a calibrated automatic lactate analyzer.

(Introduction section) (Page 3, lines 74-77.)

Since pressure-rate product is almost identical at rest and at the onset of attack in those patients, the decrease in coronary blood flow rather than increased myocardial oxygen consumption is a likely explanation for myocardial ischemia, indicating a substantial involvement with coronary microvascular spasm (MVS).

(Introduction section) (Page 3, lines 80-82.)

Since no technique is available for visualizing coronary microvessels in humans in vivo, MVS is defined as ischemic ECG changes associated with reproduction of usual chest pain in the absence of epicardial spasm (>90%) during intracoronary provocation testing.[5]

(Protocol section) (Page 6, lines 142-144.)

Since the great coronary sinus drains blood from perfusion regions of the LCA but not from the right coronary artery, evaluation of myocardial lactate production is possible only for the LCA during ACh provocation testing.[8,10]

(Protocol section) (Page 7, lines 166-169.)

Therefore, we are able to recognize the occurrence of MVS as myocardial lactate production (negative LFR) without or prior to the occurrence of angiographically apparent epicardial coronary spasm during ACh provocation testing.[3]

(Discussion section) (Page 10, lines 213-214.)

Detection of enhanced coronary vasoconstriction is possible by an additional pharmacological provocation testing with ACh or ergometrine during coronary angiography.

3. Please revise the lines of the Figure Legends also.

(Response)

Thank you very much for this valuable suggestion. In line with the Editor's comment, we have revised the descriptions in the Figure Legends as follows;

(Figure and Table legends section) (Page 9, lines 192-197.)

A setting of blood sampling for measurement of lactate concentrations in LCA and CS is shown (LAO 50°). A Judkins-left catheter was introduced into LCA (black arrow). To detect the CS orifice and visualize its whole configuration, venous phase of LCA angiography (white arrows) is useful (**A**). With reference to the CS imaging obtained at venous phase of LCA angiography, we are able to insert an Amplatz-left catheter (white outlined arrow) through right femoral vascular access into CS (white arrows) reliably and safety (**B**).

(Figure and Table legends section) (Page 9, lines 201-206.)

Coronary angiograms, ECG changes, and lactate levels during ACh provocation testing in a 56-year-old female patient with repetitive resting angina attacks. Baseline coronary angiogram of LCA and ECG findings were normal (**A**). Intracoronary 100 μg of ACh induced reproduction of her usual symptoms and marked ST-segment depression in V_2-V_4 (red arrows), but no epicardial coronary vasoconstriction was noted (**B**). Changes in myocardial lactate metabolism throughout ACh provocation testing are summarized (**C**).

4. Please ensure that abbreviations are defined at first usage.

(Response)

Thank you very much for this valuable suggestion. In line with the Editor's comment, we have defined all abbreviations when they were used for the first time.

5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.

(Response)

Thank you very much for this valuable suggestion. In line with the Editor's comment, we have removed all commercial language from our manuscript and referenced them in the Table of Materials.

6. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

Step 1: Please include any patient inclusion/exclusion criteria.

(Response)

Thank you very much for this valuable suggestion. In line with the Editor's comment, we have added some descriptions about patient inclusion criteria for measurement of myocardial lactate production. The inclusion criteria for the procedure include patients with suspected angina due to vasospasm who undergo ACh provocation testing. Thus, we have added a description in the STEP 1.1. as follows;

(Protocol section) (Page 4, lines 100-104.)

1.1. Measurement of myocardial lactate production is often performed in patients undergoing ACh provocation testing in order to diagnose VSA and/or microvascular angina (MVA) due to vasospasm. For the purposes of accuracy of those diagnoses, it is recommended to discontinue all vasoactive agents, including calcium channel blockers, long-acting nitrates, and nicorandil, at least 48 hours before catheterization study.[9]

Step 2.10: Please specify how the myocardial lactate metabolism examination was performed.

(Response)

Thank you very much for this valuable suggestion. In line with the Editor's comment, we have added a description regarding myocardial lactate metabolism examination. Recent blood gas analyzers allow us to measure various parameters including not only blood gas but also electrolytes and metabolites such as glucose, total bilirubin, creatinine, and even lactate in the same blood sample. For myocardial lactate metabolite examination, we usually use a blood gas analyzer equipped with lactate measurement function in catheterization laboratory. Thus, we have added a description in the STEP 2.10. as follows;

(Protocol section) (Page 5, lines 137-138.)

Then, measure lactate levels in those samples using blood gas analyze equipped with automatic lactate measurement function.

Step 3.2: Please mention how the coronary angiogram was performed. Also,

published references can be cited.

(Response)

Thank you very much for this valuable suggestion. In line with the Editor's comment, we have added a description regarding the timing of coronary angiograms during ACh provocation testing and have cited references as follows;

(Protocol section) (Page 6, lines 150-153.)

3.2. Administer ACh into the coronary artery in a cumulative manner (ACh 20, 50 and 100 μg in 10 mL solution) over 20 seconds with careful monitoring of blood pressure and 12-lead ECG. Perform coronary angiography when either chest pain or any ECG ST-segment change occurs, or routinely at 1 after the completion of each ACh injection.[9,11]

(References section) (Page 13, lines 99-101.)

11 Sueda, S. et al. Overview of the Acetylcholine Spasm Provocation Test. *Clin Cardiol.* **38** (7), 430-438, (2015).

7. Representative results: For the validation of the method, please provide more case studies if possible.

(Response)

Thank you very much for this valuable comment. However, since we have already demonstrated a typical case in which measurement of myocardial lactate production helped us make a diagnosis of microvascular angina due to MVS, we consider that further similar case-presentation would not be unnecessary.

8. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique

(Response)

Thank you very much for these valuable comments. In line with the Editor's comment, we have added some descriptions in the Discussion section as follows;

(Discussion section) (Page 10-11, lines 230-238.)

Basically, measurement of myocardial lactate production during ACh provocation testing is simple and safe from a technical point of view. Indeed, the procedure's success depends on the cannulation into CS. Therefore, as shown in Figure 1, it is important to identify the location of CS orifice by means of the venous phase imaging of LCA angiography before attempting to insert a catheter into CS. This process not only contributes to the ease of cannulation into CS but also prevents complications including CS dissection, perforation of CS or right atrium, and resultant cardiac tamponade. Actually, in our previous study with 198 patients who underwent the evaluation of myocardial lactate production during ACh provocation testing, no complication associated with cannulation into CS was noted.[8]

9. Please submit each figure individually as a vector image file to ensure high

resolution throughout production: (.psd, ai, .eps.).

(Response)

Thank you very much for this valuable comment. In line with the Editor's comment, we have submitted each figure in a high-resolution JPEG format.

10. Figure 1/2: Please provide scale bars in the Figures if possible.

(Response)

Thank you very much for this valuable comment. However, we are sorry that it is difficult to add scale bars in the Figures.

11. Figure 2B/C: Please include a space between the numbers and the units: ACh 20 ug instead of ACh20ug, etc.

(Response)

Thank you very much for this valuable comment. In line with the Editor's comment, we have included a space between the numbers and the units in Figure 2B and 2C.

12. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please sort the Materials Table alphabetically by the name of the material.

(Response)

Thank you very much for these valuable comments. In line with the Editor's comments, we have revised the Table for materials.

13. Please spell out journal titles in the References.

(Response)

Thank you very much for this valuable comment. In line with the Editor's comment, we have spelled out journal titles in the References.

Finally, we would like to thank the Editor for the valuable comments. We sincerely hope that our revised manuscript may again be considered for publication in the *JoVE*.