The Need for Separate Testing with Acetylcholine for the Assessment of Endothelial Dysfunction and Coronary Artery Spasm

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Abstract

Intracoronary acetylcholine (ACH) testing is clinically useful to diagnose the presence of the coronary vasomotor disorders coronary endothelial dysfunction and coronary epicardial/microvascular spasm. In Western countries, continuous intracoronary injection of ACH for 2–3 minutes without a pacemaker is the usual method, while rapid injection of ACH for 20–30 seconds with a pacemaker is the traditional procedure in Japan. Coronary microvascular spasm is often observed in Western populations, whereas coronary epicardial spasm is frequently seen in Japanese subjects. Methodological differences between Western and Japanese protocols may lead to the opposite prevalence of coronary vasomotor disorders. This article discusses the optimal method for diagnosing endothelial dysfunction and epicardial/microvascular spasm based on previous reports, and compares intracoronary ACH testing performed by Western cardiologists with that by Japanese physicians.

Keywords

Vasoreactivity testing, epicardial spasm, coronary microvascular spasm, acetylcholine, coronary endothelial dysfunction

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Intracoronary administration of acetylcholine (ACH) was found to induce paradoxical coronary vasoconstriction due to coronary endothelial dysfunction, according to Ludmer et al. in 1986.[1] They carried out incremental dose-up injection of ACH (0.03 µg, 0.3 µg, 3 µg and 30 µg) for 2 minutes with an infusion pump into the left coronary artery (LCA). In 2003, the ENCORE study used intracoronary ACH (2.16 µg, 21.6 µg and 108 µg) with an infusion pump via microcatheter for 3 minutes into the LCA to verify the presence or absence of coronary endothelial dysfunction.[2] In contrast, in 1986 Yasue et al. reported the usefulness of intracoronary ACH testing in patients with variant angina.[3] They used incremental dose-up of ACH (20 µg, 50 µg and 100 µg into the LCA and 20 µg and 50 µg into the right coronary artery [RCA]) for 20–30 seconds with manual injection. The sensitivity and specificity of ACH testing for patients with variant angina were found to be acceptable.[3] Intracoronary ACH testing is clinically used for the investigation of coronary endothelial dysfunction and coronary spasm; however, the procedures and doses involved vary worldwide. In this article, we summarise the information on intracoronary ACH testing with the aim of determining the optimal ACH procedure for verifying the presence or absence of coronary endothelial dysfunction and coronary spasm.

Vasoreactivity Testing for Coronary Endothelial Dysfunction

Furchgott and Zawadzki reported on the essential role of endothelial cells in the relaxation of arterial smooth muscle by ACH *in vitro*.[4] If coronary arteries have normal endothelial function without any atherosclerotic lesions, intracoronary injection of ACH dilates the coronary artery. However, if coronary arteries have abnormal endothelial function due to coronary atherosclerosis, intracoronary injection of ACH constricts the coronary artery. As shown in *Table 1*, the majority of researchers have used relatively low doses of ACH (0.36 µg, 3.6 µg and 36 µg) for 2–3 minutes.[5–10] Intracoronary ACH is injected at a constant flow rate using pump infusion without a pacemaker, and the target coronary artery is usually the left anterior descending artery.[11–13] In Japan, there are few reports on coronary endothelial dysfunction.[14,15]

Inconsistency of Intracoronary ACH Testing in the Clinic

Physicians use intracoronary ACH testing to verify the presence of coronary spasm or coronary endothelial dysfunction. Low doses of ACH for 2–3 minutes' continuous injection are used for the diagnosis of coronary endothelial function, while bolus injection of high-dose ACH for 20–30 seconds is used to investigate the presence of coronary epicardial spasm/coronary microvascular spasm.[16] However, physicians may perform additional ACH testing arbitrarily (*Tables 1–3*). Furthermore, the majority of physicians do not perform vasoreactivity testing on both coronary arteries.[7,16] They perform vasoreactivity testing mainly into the left anterior descending artery. If they obtain negative results for intracoronary ACH testing in the LCA, they then carry out intracoronary injection of ACH into the RCA whenever possible.

Table 1: Intracoronary Acetylcholine Testing for Coronary Endothelial Dysfunction

	Year	Method	LCA Dose (µg)	RCA Dose (µg)	Injection Time	CAG Timing	Interval	Other
Endothelial function								
Ludmer et al.[1]	1986	Pump	0.03/0.3/3/30	None	2 min	Not described	Not described	No pacing
WISE[5]	1999	Pump	0.2/21	None	3 min	3 min	5 min	No pacing
ENCORE I[2]	2003	Pump	2.16/21.6/108	None	3 min	Not described	Not described	No pacing
ENCORE II[45]	2009	Pump	2.16/21.6/108	None	3 min	Not described	Not described	No pacing
WISE (Wei et al.)[6]	2012	Pump	0.36/3.64/36.4	None	3 min	3 min	5 min	No pacing
Lee et al.[11]	2015	Manual	50/100	None	>2–3 min	2–3 min	Not described	No pacing
CorMicA trial[7]	2018	Manual	0.36/3.64/36.4	None	2 min	Not described	Not described	No pacing
Widmer et al.[8]	2019	Pump	0.5/5/50	None	3 min	Not described	Not described	No pacing
EAPCI[9]	2020	Pump	0.36/3.64/36.4	None	Not described	Not described	Not described	No pacing
Pargaonkar et al.[12]	2020	Manual	20/50/100/200	None	>1 min	Not described	Not described	No pacing
COVADIS 2020[10]	2020	Pump	0.36/3.64/36.4	None	2 min	Not described	Not described	No pacing
Gutierrez et al.[13]	2021	Manual	2/20/100	None	2–3 min	Not described	Not described	No pacing
Egashira et al.[14]	1995	Manual	2/6//20/60	None	2 min	Not described	Not described	No pacing
Akiyama et al.[15]	2021	Manual	10/30/100	None	Not described	Not described	Not described	No pacing

CAG = coronary angiography; LCA = left coronary artery; RCA = right coronary artery.

Duration of ACH Injection: 20 Seconds versus 180 Seconds

The duration of ACH injection plays an important role in the type of spasm provoked. Given the same doses of ACH in the same patients, rapid injection of ACH provoked more cases of epicardial spasm than moderate administration of ACH.[17] Provoked spasm incidence by a 20-second injection of ACH was significantly higher than that by a 180-second ACH injection (73.3%, 22/30 versus 33.3%, 10/30, $p<0.05$).[17] As shown in *Figure 1*, intracoronary injection of ACH for 20 seconds provoked typical epicardial spasm but that for 180 seconds did not (case 1), whereas in case 2, intracoronary injection of ACH for both 20 seconds and for 180 seconds produced the same coronary responses. Cardiologists should understand the clinical differences underlying the coronary responses between the two procedures, even in the same patients.

Transition for Ideal Vasoreactivity Testing of ACH

In 2014, Ong et al. reported on an incremental dose-up of 2 µg, 20 µg, 100 µg and 200 µg ACH manually infused over a period of 3 minutes into the LCA via angiographic catheter.[18] The ACH doses in their protocol were derived from the multicentre ENCORE study (*Supplementary Figure 1*).[2] In the ENCORE study, the dose for the left anterior descending artery and for the left circumflex artery was 100 µg into each vessel injected via selective catheter. For practical reasons, in the Ong et al. study the ACH injection was performed unselectively via the diagnostic catheter into the LCA with a maximum dose of 200 µg. In patients who remained asymptomatic and had no diagnostic ST-segment changes during LCA ACH infusion, 80 µg ACH was injected into the RCA.[18] In the CASPAR study in 2008, incremental doses of 2 µg, 20 µg and 100 µg ACH were injected into the LCA via the diagnostic catheter for 3 minutes each.[19] However, in 2016, Ong et al. reported the use of the same ACH doses with an injection duration of 20 seconds instead of 3 minutes without a pacemaker.[20] The maximum ACH dose and the injection time in their study varied from 100 µg ACH to 200 µg ACH and from 3 minutes to 20 seconds, respectively.

In contrast, we performed intracoronary ACH testing from 1991 based on the Japanese Circulation Society (JCS) guidelines.[21] Incremental dose-up of 20 µg and 50 µg into the RCA and of 20 µg, 50 µg and 100 µg into the LCA was injected for 20 seconds with a pacemaker. We used a maximum ACH dose of 80 µg into the RCA from 1993, and a maximum ACH dose of 200 µg into the LCA from 2012.[22]

We have been performing intracoronary ACH testing for more than 30 years, but currently, there is no established global standard for ACH testing. Therefore, an important goal for the future is to establish optimum, unified recommendations for ACH testing for coronary spasm and coronary endothelial dysfunction.

Vasoreactivity Testing for Coronary Epicardial Spasm

The intracoronary injection time for ACH in Japanese reports is less than 30 seconds, while in the majority of Western reports it is over 3 minutes (*Table 2*).[23–39] Furthermore, back-up pacing was necessary in Japanese reports, whereas in Western reports there was no back-up pacing during vasoreactivity testing of ACH. There are obvious methodological differences between Japanese and Western studies, and hence a unified method is needed to verify the presence or absence of coronary epicardial spasm. In the Western reports, if a positive epicardial spasm was not provoked in the LCA, intracoronary ACH was administered into the RCA. The main target coronary artery is the LCA for Western cardiologists, while Japanese cardiologists perform tests for both coronary arteries if possible. The definition of positive epicardial spasm was similar between Western and Japanese reports.

Vasoreactivity Testing for Coronary Microvascular Spasm

As shown in *Table 3*, the majority of researchers used the same methods to diagnose the presence or absence of epicardial spasm and coronary microvascular spasm.[40] There are no recommendations regarding methodological issues in the COVADIS group 2017/18 reports.[41,42] However, COVADIS reports in 2020 provided recommendations on ACH doses for the first time.[10] They recommended intracoronary injection of ACH 100 µg into the LCA and ACH 50 µg into the RCA for 20 seconds without a pacemaker. Compared with Western reports, the ACH intracoronary injection time is remarkably short in Japanese reports. Back-up pacemaker

Table 2: Intracoronary Acetylcholine Testing for Vasospastic Angina

	Year	Method	LCA Dose (µg)	RCA Dose (µg)	Injection Time	CAG Timing	Interval	Other	VSA % (n)
VSA Western									
Pristipino et al.[33]	2000	Manual	25/50/100	25/50	20 s	Not described	5 min	No pacing	16 (3/19)
Kim et al.[34]	2007	Manual	20/50/100	None	1 min	Not described	Not described	No pacing	77 (62/81)
Ong et al. (CASPAR)[19]	2008	Manual	2/20/100	80	3 min	Not described	Not described	No pacing	49 (42/86)
Ong et al. (ACOVA)[35]	2012	Manual	2/20/100/200	80	>3 min	Not described	Not described	No pacing	28 (35/124)
Schoenenberger et al.[36]	2013	Manual	64	64	>3.5 min	Not described	Not described	No pacing	20 (145/718)
Ong et al.[18]	2014	Manual	2/20/100/200	80	>3 min	Not described	Not described	No pacing	33 (283/847)
Ong et al.[20]	2016	Manual	2/20/100/200	80	20 s	Not described	Not described	No pacing	
Aziz et al.[16]	2017	Manual	2/20/100/200	80	>3 min	Not described	1 min	No pacing	26 (355/1,379)
COVADIS 2017[41]	2017	Manual	Not described	Not described	Not described	Not described	Not described	No pacing	
CorMicA[7]	2018	Manual	100	50	bolus	Not described	Not described	No pacing	37 (56/151)
Montone et al.[37]	2018	Manual	2/20/50/100/200	20/50/80	>3 min	Not described	2–3 min	No pacing	30 (24/80)
Widmer et al.[8]	2019	Manual	100	None	20–30 s	Not described	Not described	No pacing	
Seitz et al.[38]	2020	Manual	2/20/100/200	80	>3 min	Not described	Not described	No pacing	32 (177/552)
EAPCI[9]	2020	Manual	2/20/100/200	50/80	>3 min			No pacing	
COVADIS 2020[10]	2020	Manual	100	50	20 s	Not described	Not described	No pacing	
Jansen et al.[39]	2021	Manual	2/20/100/200	None	>1–3 min	Not described	Not described	No pacing	44 (118/266)
Gutierrez et al.[13]	2021	Manual	2/20/100	2/20/50	20 s	Not described	Not described	No pacing	
VSA Japanese									
Yasue et al.[3]	1986	Manual	10/20/30/50/80/100	10/20/30/50/80/100	Not described	3 min	Not described	Back-up pacing	93 (25/27)
Yasue et al.[3]	1986	Manual	20/50/100	20/50	Not described	3 min	Not described	Back-up pacing	93 (25/27)
Okumura et al.[23]	1988	Manual	20/50/100	20/50	Not described	1.5–3 min	Not described	Back-up pacing	97 (32/33)
Okumura et al.[24]	1988	Manual	20/50/100	20/50	>20 s	1.5–3 min	>5 min	Back-up pacing	90 (63/70)
Mohri et al.[25]	1998	Manual	10/30/100	5/15/50	>30 s	1 min	2 min	Back-up pacing	51 (57/111)
Sueda et al.[26]	1999	Manual	20/50/100	20/50/80	>20 s	<3 min	3 min	Back-up pacing	32 (221/685)
Wakabayashi et al.[27]	2008	Manual	50/100	20/50	>20 s	Not described	5 min	Back-up pacing	73 (174/240)
Ohba et al.[28]	2012	Manual	20/50/100	50	>30 s	1 min	5 min	Back-up pacing	58 (216/370)
Satoh et al.[29]	2013	Manual	50/100	25/50	15 s	1 min	Not described	Back-up pacing	54 (70/130)
JCS guidelines[21]	2014	Manual	20/50/100	20/50	>20 s	1 min	5 min	Back-up pacing	
Odaka et al.[30]	2017	Manual	20/50/100	20/50	>30 s	1 min	5 min	Back-up pacing	73 (145/198)
Suda et al.[31]	2019	Manual	20/50/100	20/50	>30 s	1 min	5 min	Back-up pacing	68 (128/187)
Sueda & Sakaue[32]	2021	Manual	20/50/100/200	20/50/80	>20 s	1–2 min	3 min	Back-up pacing	44 (329/746)

CAG = coronary angiography; LCA = left coronary artery; RCA = right coronary artery; VSA = vasospastic angina.

insertion is necessary for ACH testing in Japan, while we could not find any mention of the procedure under pacemaker in Western reports.[43] The definition of positive coronary microvascular spasm in Western reports is not different from that in Japanese reports.

Initial Examination: Physiological Functional Tests versus Vasoreactivity Testing

Western guidelines recommend coronary physiological functional measurements before vasoreactivity testing.[9] However, Japanese physicians recommend vasoreactivity testing first, before coronary physiological functional measurements.[43] Furthermore, Western cardiologists reported that the effect of intracoronary injection of <200 µg nitroglycerine had almost completely disappeared 10 minutes later, possibly due to the short half-life.[44] If ACH testing is performed first, then resting flow and coronary flow reserve assessment may be inaccurate, particularly after a positive vasospasm test.[17] However, JCS guidelines recommend at least 48 hours' cessation of coronary vasodilators before coronary vasoreactivity testing. Western guidelines place considerable weight on the identification of coronary microvascular dysfunction, whereas Japanese researchers place importance on diagnosing the presence of epicardial spasm.

These methodological and ethnic differences may be contributing to the disparity in diagnostic strategies. Therefore, if the aim is to diagnose coronary epicardial spasm or coronary microvascular spasm, we suggest that ACH vasoreactivity testing be done first, before the assessment of coronary physiological functioning.

Need for Standardised Vasoreactivity Testing for Coronary Epicardial and Coronary Microvascular Spasm

Although there are ethnic and racial differences in epicardial spasm and

Table 3: Intracoronary Acetylcholine Testing for Coronary Microvascular Spasm

	Year	Method	LCA Dose (µg)	RCA Dose (µg)	Injection Time	CAG Timing	Interval	Other	CMS % (n)
CMS Western									
Ong et al. (ACOVA)[35]	2012	Manual	2/20/100/200	80	>3 min	Not described	Not described	No pacing	34 (42/124)
Schoenenberger et al.[36]	2013	Manual	64	64	>3.5 min	Not described	Not described	No pacing	32 (233/718)
Ong et al.[18]	2014	Manual	2/20/100/200	80	>3 min	Not described	Not described	No pacing	24 (205/847)
Ong et al.[20]	2016	Manual	2/20/100/200	80	20 s	Not described	Not described	No pacing	
Aziz et al.[16]	2017	Manual	2/20/100/200	80	>3 min	Not described	1 min	No pacing	33 (458/1,379)
COVADIS 2018[42]	2018	Manual	Not described	Not described	Not described	Not described	Not described	No pacing	
CorMicA trial[7]	2018	Manual	100	50	Bolus	Not described	Not described	No pacing	72 (109/151)
Montone et al.[37]	2018	Manual	2/20/50/100/200	20/50/80	>3 min	Not described	2–3 min	No pacing	16 (13/80)
Seitz et al.[38]	2020	Manual	2/20/100/200	80	>3 min	Not described	Not described	No pacing	27 (148/552)
EAPCI[9]	2020	Manual	2/20/100/200	50/80	>3 min	Not described	Not described	No pacing	
COVADIS 2020[10]	2020	Manual	100	50	20 s	Not described	Not described	No pacing	
Jansen et al.[39]	2021	Manual	2/20/100/200	None	>1–3 min	Not described	Not described	No pacing	38 (102/266)
Gutierrez et al.[13]	2021	Manual	2/20/100	2/20/50	20 s	Not described	Not described	No pacing	
CMS Japanese									
Mohri et al.[25]	1998	Manual	10/30/100	5/15/50	>30 s	1 min	2 min	Back-up pacing	26 (29/111)
Sun et al.[40]	2002	Manual	10/30/100	None	>30 s	Not described	Not described	Back-up pacing	25 (14/55)
Ohba et al.[28]	2012	Manual	20/50/100	50	>30 s	1 min	5 min	Back-up pacing	14 (50/370)
Odaka et al.[30]	2017	Manual	20/50/100	20/50	>30 s	1 min	5 min	Back-up pacing	33 (66/198)
Suda et al.[31]	2019	Manual	20/50/100	20/50	>30 s	1 min	5 min	Back-up pacing	12 (22/187)
Sueda and Sakaue[32]	2021	Manual	20/50/100/200	20/50/80	>20 s	1–2 min	3 min	Back-up pacing	5 (40/746)

CAG = coronary angiography; CMS = coronary microvascular dysfunction; LCA = left coronary artery; RCA = right coronary artery.

coronary microvascular spasm, unified diagnostic testing worldwide is essential for patients with coronary vasomotor disorders, especially given that the definition of positive coronary microvascular spasm and of coronary microvascular dysfunction is not different between Western and Japanese reports. The challenge is, therefore, to establish a unified procedure for diagnosing epicardial spasm and coronary microvascular spasm.

Complications during Intracoronary ACH Testing

In the ENCORE II study, one patient died during intracoronary 3 minute ACH infusion for detecting endothelial function due to acute MI possibly related to ACH.[45] Bradycardia has often been observed in Western reports, possibly due to the lack of back-up pacing, while the prevalence of ventricular tachycardia or ventricular fibrillation is remarkably higher during ACH testing in Japanese reports (*Supplementary Table 1*). The occurrence of paroxysmal AF in Japanese reports is markedly higher than that in Western reports.[18,22] Although we could not find a full list of the various complications in each report, there seem to be no irreversible complications during intracoronary ACH testing in the recent reports.[18,32,37]

Given that the COVADIS reports and the JCS guidelines recommend vasoreactivity testing as class 1, trained physicians can perform intracoronary ACH vasoreactivity testing without any complications. However, the European Society of Cardiology (ESC) and American College of Cardiology/American Heart Association (ACC/AHA) guidelines still recommend vasoreactivity testing as class 2b.[46,47]

Future Recommendations

Global guidelines for the diagnosis of epicardial spasm, coronary microvascular spasm and coronary microvascular dysfunction are necessary, as are unified diagnostic strategies for use in the cardiac catheterisation laboratory. As shown in *Figure 2*, the proposed ideal diagnostic interventional procedures may be cumbersome and time-consuming. A procedure time of approximately 1 hour is necessary to diagnose coronary spasm and coronary endothelial dysfunction. Furthermore, radiation exposure and contrast medium due to repetitive coronary angiography are serious problems.

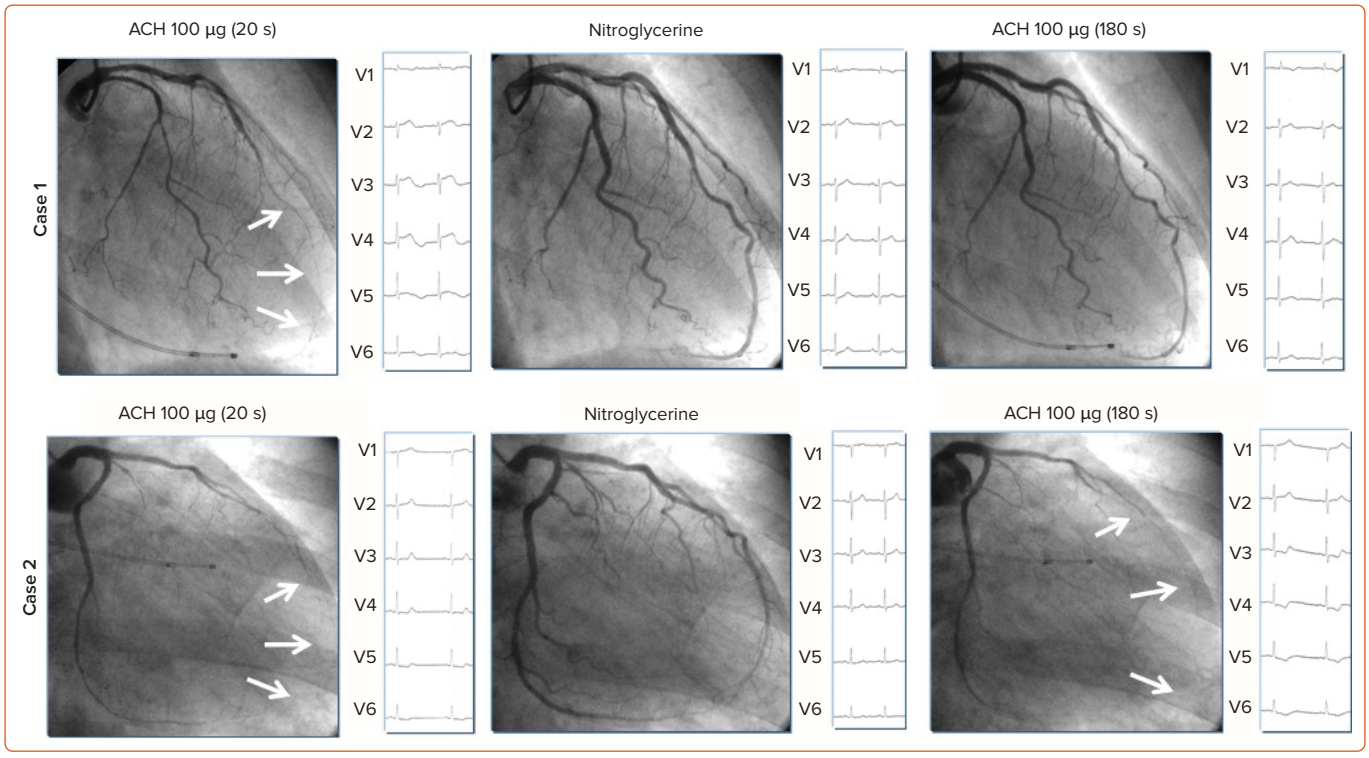
The cardiologist may perform culling vasoreactivity testing of ACH if patients have no coronary constriction after the injection of low-dose ACH. However, if cardiologists around the world perform this diagnostic interventional procedure carefully in the cardiac catheterisation laboratory, they may reach a satisfactory diagnosis for each patient.

Global guidelines should be established to unify the diagnosis of epicardial spasm, coronary microvascular spasm and coronary microvascular dysfunction, and the COVADIS, ESC, AHA/ACC, ACI-SEC (Interventional Cardiology Association of the Spanish Society of Cardiology) and JCS guidelines.

Conclusion

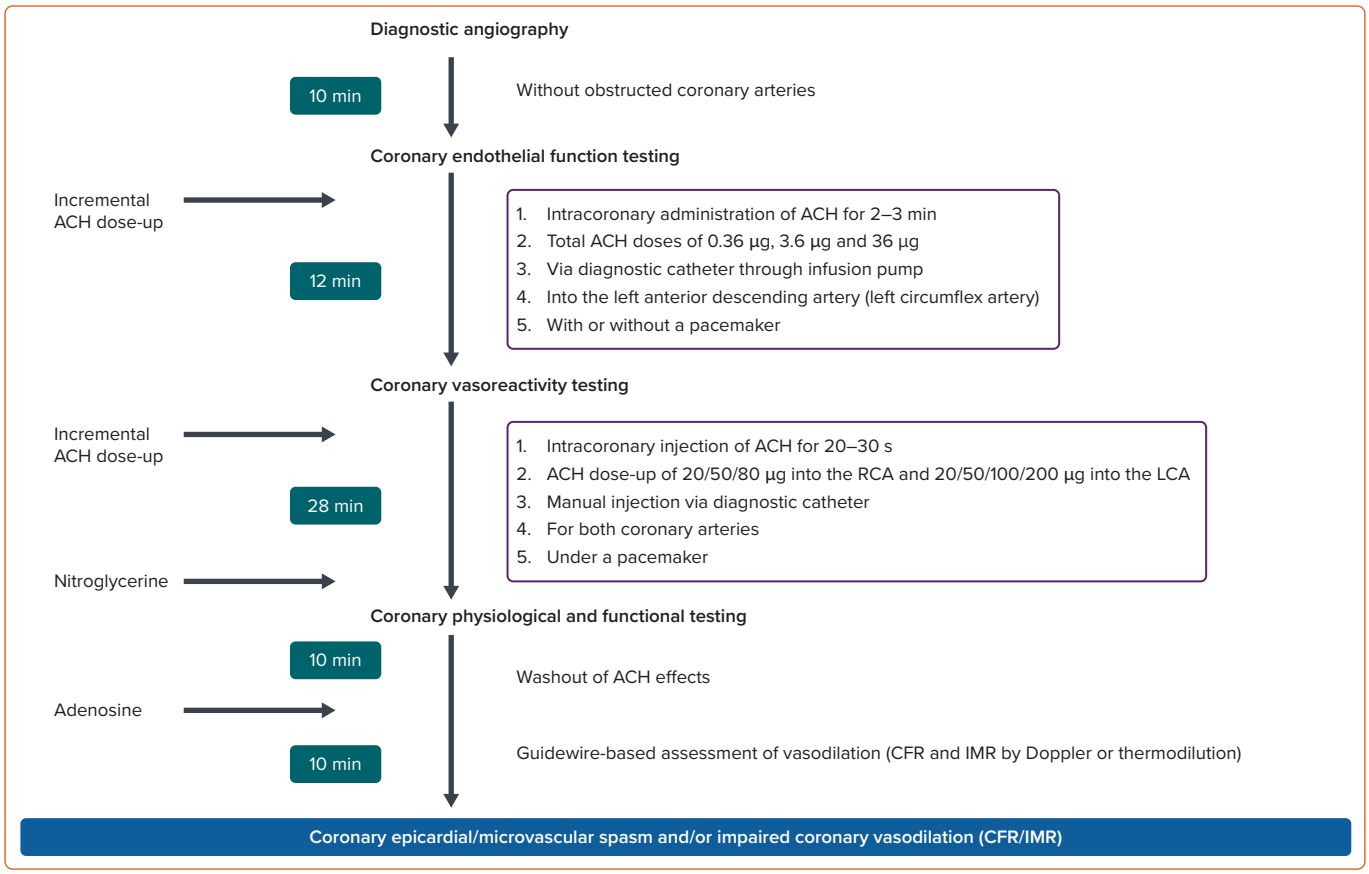
Vasoreactivity testing with ACH has two diagnostic functions: to identify coronary endothelial function and to investigate coronary microvascular spasm and epicardial spasm. However, each physician uses a different modified ACH protocol to diagnose epicardial spasm, coronary microvascular spasm and coronary endothelial dysfunction. Unified ACH testing for coronary spasm and endothelial function is, therefore, essential for cardiologists. ❑

Figure 1: Vasoreactivity Testing: Acetylcholine Injection of 20 s versus 180 s



Case 1: A 55-year-old woman who complained of chest pain at rest. Intracoronary injection of ACH 100 µg for 20 s into the left coronary artery provoked diffuse spasm at the distal left anterior descending artery accompanied with usual chest pain and ST-segment elevation in anterior leads. However, intracoronary ACH 100 µg for 180 s produced no epicardial spasm, no usual chest pain, nor ischaemic ECG changes. After the administration of nitroglycerine, non-obstructive coronary artery was observed in the left coronary artery. Case 2: A 79-year-old woman whose chief complaint was resting angina. Intracoronary injection of ACH 100 µg for 20 s and for 180 s produced diffuse spasm at the mid and distal left anterior descending artery accompanied with usual chest pain and ischaemic ECG changes. Non-obstructed coronary artery was confirmed after the injection of nitroglycerine in the left coronary artery. ACH = acetylcholine.

Figure 2: Proposed Interventional Diagnostic Strategy Protocol



ACH = acetylcholine; CFR = coronary flow reserve; IMR = index of microcirculatory resistance; LCA = left coronary artery; RCA = right coronary artery.

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