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The interventional diagnostic procedure for assessment of coronary vasomotor disorders: acetylcholine testing followed by adenosine testing

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TITLE:

Testing Acetylcholine Followed by Adenosine for Interventional Diagnosis of Coronary Vasomotor Disorders

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KEYWORDS:

coronary artery spasm, angina pectoris, acetylcholine testing, coronary flow reserve, adenosine, microvascular dysfunction.

SUMMARY:

Coronary vasomotion disorders represent frequent functional causes of angina in patients with unobstructed coronaries. The underlying mechanism of angina (endotype) in these patients can be determined by a comprehensive invasive diagnostic procedure based on acetylcholine provocation testing followed by Doppler-derived assessment of the coronary flow reserve and microvascular resistance.

ABSTRACT:

More than 50% of patients with signs and symptoms of myocardial ischemia undergoing coronary angiography have unobstructed coronary arteries. Coronary vasomotor disorders (impaired vasodilatation and/or enhanced vasoconstriction/spasm) represent important functional causes for such a clinical presentation. Although impaired vasodilatation may be assessed with non-invasive techniques such as positron emission tomography or cardiac magnetic resonance imaging, there is currently no reliable non-invasive technique for the diagnosis of coronary spasm available. Thus, invasive diagnostic procedures (IDP) have been developed for the diagnosis of coronary vasomotor disorders including spasm testing as well as assessment of coronary vasodilatation. The identification of the underlying type of disorder (so called endotype) allows the initiation of targeted pharmacological treatments. Despite the fact that such an approach is recommended by the current European Society of Cardiology guidelines for the management of chronic coronary syndromes based on the CorMicA study, comparability of results as well as

multicenter trials are currently hampered by major differences in institutional protocols for coronary functional testing. This article describes a comprehensive IDP protocol including intracoronary acetylcholine provocation testing for diagnosis of epicardial/microvascular spasm, followed by Doppler wire-based assessment of coronary flow reserve (CFR) and hyperemic microvascular resistance (HMR) in search of coronary vasodilatory impairment.

INTRODUCTION:

In recent years interventional cardiology has made substantial progress in various areas. This not only comprises interventional treatment of the heart valves using transcatheter aortic valve replacement and edge-to-edge repair of the mitral and tricuspid valve, but also coronary interventions[1–6]. Among the latter are advances in techniques for treatment of chronic total occlusions as well as calcified lesions using rotablation and shock wave therapy. In addition to these rather structural coronary interventional procedures invasive diagnostic procedures (IDP) have now been established in search of functional coronary disorders (i.e., coronary spasm and microvascular dysfunction)[7]. The latter comprise a heterogeneous group of conditions frequently but not exclusively occurring in patients with angina pectoris and unobstructed coronary arteries. The main mechanisms underlying these vasomotor disorders are impaired coronary vasodilatation, enhanced vasoconstriction, and spasm as well as enhanced coronary microvascular resistance. The latter is often due to obstructive microvascular disease[8]. Anatomically, coronary vasomotor disorders may occur in the epicardial arteries, the coronary microcirculation or both. The Coronary Vasomotor Disorders International Study group (COVADIS) has published definitions for the diagnosis of these disorders[9,10] and recent guidelines of the European Society of Cardiology (ESC) on the management of patients with chronic coronary syndrome have made recommendations for adequate patient assessment depending on the clinical condition[11]. Moreover, recent publications have delineated the various endotypes that can be derived from an IDP[12,13]. Such an approach has a benefit for the individual patient as randomized studies have shown better quality of life in patients undergoing an IDP followed by stratified medical therapy according to the test result compared to usual care by the general practitioner[14]. Currently, there is a debate about the most appropriate protocol for testing of such vasomotor disorders. The aim of this article is to describe a protocol where acetylcholine (ACh) provocation testing in search of coronary spasm is followed by Doppler-wire-based assessment of coronary flow reserve (CFR) and hyperemic microvascular resistance (HMR) using adenosine.

PROTOCOL:

Intracoronary ACh testing has been approved by the local ethics committee and the protocol follows the guidelines of our institution for human research. A previous JOVE article covered a protocol showing preparation of the ACh solutions as well as preparation of the syringes for intracoronary injection of ACh[15].

1. Preparation of the ACh solutions and preparation of the syringes for intracoronary injection of Ach

1.1. Please refer to a previously published JoVE article[15].

2. Preparation of adenosine solution for intracoronary injection

2.1. Take 1 ampoule of 6 mg adenosine (with 2 mL solvent) into a syringe (this corresponds to a dose of 3 mg/mL).

2.2. Add the 6 mg of adenosine to 100 mL of 0.9% sodium chloride solution and mix gently.

2.3. Fill a 10 mL syringe with 3.5 mL of the adenosine solution (approximately 200 µg of adenosine).

2.4. Perform the last step 3 times for preparation of 3 injections.

3. Diagnostic coronary angiography

3.1. Depending on arterial access route, inject local anaesthesia either in proximity to the right femoral artery (usually 15 mL of mepivacaine) or in proximity to the right radial artery (usually 2 mL of mepivacaine).

3.2. To confirm the success of local anaesthesia, prick the anesthetized-skin with the needle and ask the patient if pain is still present.

3.3. Puncture the artery according to the Seldinger technique and insert the sheath (usually 5F). If possible, omit radial spasm prophylaxis in patients undergoing planned IDP. Perform coronary angiography under sterile conditions.

3.4. Introduce the diagnostic catheter over a J-tipped wire through the radial artery sheath to the ascending aorta and advance it to the aortic root.

3.5. Give 5000 IU of heparin.

3.6. Engage the diagnostic catheter into the ostium of the right (RCA) and subsequently of the left coronary artery (LCA). Inject 2 mL of contrast to confirm correct positioning of the catheter.

3.7. Perform coronary angiography in different views using manual injections of approximately 10 mL of contrast agent under fluoroscopy to visualize the coronary arteries.

NOTE: Usually LAO 40° and RAO 35° are used for the RCA and LAO 45°/ CRAN 25°, RAO 30°/ CRAN 30° and RAO 20°/ CAUD 30° are used for the LCA.

4. Preparations for the IDP

4.1. Aa a prerequisite for the IDP, exclude any epicardial stenosis of >50% on visual

assessment.

NOTE: The default artery for the IDP is the LCA as it allows the examination of the two vessels (left anterior descending artery (LAD) and left circumflex artery (LCX)) at the same time.

4.2. Place a guiding catheter suitable for the LCA into the left main (this can be 5F or 6F, choice of the catheter depends on the patient's anatomy).

4.3. Give another 5000 IU of heparin.

4.4. Advance the Doppler flow-/pressure-wire through the guiding catheter into the left main artery.

4.5. After flushing, calibrate the catheter with the fractional flow reserve (FFR) sensor (localized either tip-adjacent or 1.5 cm offset depending on the wire type in the left main (press **Norm** on the software of the computer system), to avoid any contrast in the catheter the Doppler flow-/pressure-wire.

4.6. Place the tip of the wire is in the proximal-mid portion of the vessel (usually LAD). Perform angiography to record wire position.

4.7. Assess and optimize the Doppler and ECG signal quality, if needed.

NOTE: This can be done by turning or pulling the wire in order to optimize the wire position. There is also the possibility for fine tuning of the Doppler-signal within the system settings (e.g., optimal tracing and scaling of ECG- and Doppler-signals, wall filter adjustment, etc.).

4.8. Once a good signal is obtained, press **Record** to record the signals on the system. The patient is now ready for the IDP.

5. Carrying out the IDP

5.1. Inject 6 mL of the lowest ACh concentration (0.36 µg/mL) into the LCA (~ 2 µg of ACh) within 20 s. Flush with 3-4 mL of saline. Perform continuous 12-lead ECG monitoring and ask the patient for recognizable anginal symptoms (e.g., chest pain, dyspnoea). Observe the Doppler-signal curves and record the average peak velocity (APV) during ACh injection.

5.2. Perform coronary angiography of the LCA after ACh injection by manual injection of approximately 10 mL of contrast agent through the catheter. After every ACh dose, record and print the 12-lead-ECG. Ask the patient for recognizable anginal symptoms. Give a 1 min pause between every dose.

NOTE: Usually a RAO 20°/ CAUD 30° projection is the best projection for ACh testing.

5.3. Inject 6 mL of the medium ACh concentration (3.6 µg/mL) into the LCA (~ 20 µg of ACh). Inject within 20 s with continuous monitoring of the 12-lead ECG and the patient's symptoms. Flush with 3-4 mL of saline. Observe the Doppler-signal curves and record the APV during ACh injection. Perform coronary angiography of the LCA after the 6 mL injection of ACh as mentioned above.

5.4. Inject 5.5 mL of the highest ACh concentration (18 µg/mL) into the LCA (~ 100 µg of ACh). Inject within 20 s with continuous monitoring of the ECG and the patient's symptoms. Flush with 3-4 mL of saline. Observe the Doppler-signal curves and record the APV during ACh injection. Repeat coronary angiography of the LCA as described above.

NOTE: In most patients with coronary spasm, symptom reproduction, ECG changes or epicardial vasoconstriction develop at this dose. If bradycardia occurs during ACh injection, this can be resolved by slowing down the speed of the manual ACh injection. A slower injection over a period of 3 min compared to the 20 s injection is also feasible.

5.5. If no epicardial spasm (i.e., > 90% vasoconstriction) occurs at the 100 µg dose continue with the 200 µg of ACh dose (11 mL of the highest ACh concentration (18 µg/mL). Inject within 20 s with continuous monitoring of the ECG and the patient's symptoms. Flush with 3-4 mL of saline. Observe the Doppler-signal curves and record the APV during ACh injection. Repeat coronary angiography of the LCA.

NOTE: Slow down the speed of the manual ACh injection if bradycardia occurs as mentioned above.

5.6. Inject 200 µg of nitroglycerine into the LCA at the end of the ACh test or when severe symptoms (i.e., severe angina or dyspnoea), ischemic ECG shifts or epicardial spasm occurs. Perform coronary angiography of the LCA after approximately one minute to document reversion of spasm.

5.7. After the APV returns to the baseline and ECG as well as patient’s symptoms become normal, perform the next step (i.e., CFR, HMR assessment).

5.8. Press **Base** to capture baseline values of APV as well as distal (Pd) and aortic (Pa) pressure.

5.9. Quickly inject a bolus of 3.5 mL of the adenosine solution into the LCA (~ 200 µg of adenosine) followed by a brief saline flush (10 mL). Press the **Peak search** button 3 heart beats after the injection to Initiate peak search (maximal APV and minimal Pd, in order to avoid influences of flushing. The system calculates and displays the values for FFR, CFR and HMR.

NOTE: The intracoronary injection of adenosine is well tolerated by the patients with only few side effects such as palpitations.

5.10. Repeat the previous steps (5.8 & 5.9) until 2 concurring measurements have been successfully done. Calculate the mean FFR/CFR/HMR from the values of the measurements.

5.11. Pull back the Doppler flow-/pressure-wire into the left main to check for pressure drift.

5.12. Pull out the Doppler flow-/pressure-wire and take a final image of the LCA to document that no vessel injury has occurred.

REPRESENTATIVE RESULTS:

According to the diagnostic criteria suggested by COVADIS[9], vasospastic angina can be diagnosed if the following criteria apply during ACh provocation testing: transient ECG changes indicating ischemia, reproduction of the patient´s usual anginal symptoms and > 90% vasoconstriction of epicardial vessels as confirmed during coronary angiography (**Figure 2**).

Spasm of the coronary microvasculature can be diagnosed if the patient´s symptoms and ischemic ECG alterations occur during provocation testing in the absence of epicardial vasospasm[10] (**Figure 3**).

Impaired microvascular vasodilatation can be diagnosed by interpreting the CFR and HMR measurements following adenosine injections. Depending on the cut-off values applied, a reduced CFR is defined as < 2.0[12,13] or ≤ 2.5[16], respectively (**Figure 4**). For HMR, data on optimal cut-off values is scarce, but an increased microvascular resistance is currently defined as a HMR > 1.9[17] or >2.4[7] (**Figure 5**).

FIGURE AND TABLE LEGENDS:

Figure 1: Flow chart of the Invasive Diagnostic Procedure. After exclusion of any epicardial stenosis during diagnostic angiography, the vasoconstrictive potential of coronary arteries is tested by intracoronary injection of incremental doses of ACh. After provocation testing, assessment of vasodilatation by intracoronary injection of adenosine, followed by measurement of CFR and HMR.

Figure 2: **58-year old female patient with diffuse epicardial spasm during ACh provocation testing**. **A**) Baseline measurement before ACh injection showing neither stenosis nor ischemic ECG changes. **B**) Diffuse epicardial spasm after intracoronary injection of 200 µg ACh into the left main, accompanied by T-inversion in lead aVL and descending ST-depression in leads I and V_2-V_6 (red arrows) during reproduction of patient´s symptoms.

Figure 3: **61-year old female patient with microvascular spasm during ACh provocation testing.** **A**) Baseline measurement before ACh injection showing neither stenosis nor ischemic ECG changes. **B**) Minor vasoconstriction of epicardial vessels after intracoronary injection of 100 µg ACh into the left main. The patient experienced her usual symptoms, going along with ST-segment depression in leads II, V_3-V_6 (red arrows).

Figure 4: **Assessment of vasodilatation by measurement of CFR.** After injection of adenosine,

APV increased insufficiently from 36 cm/s at rest (**A**) by approx. 50% to 55 cm/s (**B**), leading to a pathological CFR of 1.5. Measurements to be performed until two concurring readings are obtained (additional measurements not shown); CFR equates to mean of measurements.

Figure 5: **Assessment of vasodilatation by measurement of HMR.** For HMR calculation, average peak velocity (APV) and distal coronary artery pressure (P_d) are measured after injection of adenosine, leading to a pathological HMR of 2.3. Measurements to be performed until two concurring readings are obtained (additional measurements not shown); HMR equates to mean of measurements.

DISCUSSION:

Management of patients with angina and unobstructed coronary arteries is often demanding and sometimes frustrating. An important step during the work-up of these patients is that the underlying pathophysiological mechanism(s) for the patient's symptoms are adequately investigated. This is challenging as often not only one mechanism is responsible and various aetiologies including cardiac and non-cardiac as well as coronary and non-coronary need to be assessed.

Frequently patients with chest pain of unknown origin are scheduled for invasive diagnostic coronary angiography in search of stenosing epicardial coronary disease. Several studies have shown that despite convincing symptoms and abnormal non-invasive stress tests such patients have unobstructed coronary arteries in more than 50% of cases[12,18]. Although it is correct that the yield of patients with relevant epicardial stenoses needs to be improved it should not be neglected that functional coronary disorders can be responsible for such a clinical presentation. We and others have shown that impaired coronary vasodilatation and/or coronary spasm may account for more than 60% of such cases[12,18]. Establishing a diagnosis in these often unsettled patients represents an important step in patient management. Thus, it is important to take the opportunity of the diagnostic coronary angiography for further testing. Although this may prolong catheter laboratory time for approximately 30 minutes, establishing a diagnosis may prevent patients from coming back for repeated diagnostic angiography in the future and allow the initiation of targeted pharmacological treatments.

In this context several protocols for an IDP have been developed over the last years. This involves assessment of vasoconstriction and spasm as well as vasodilatation and microvascular resistance. Some centres have added additional assessments to their protocol including measurements of lactate concentrations in coronary sinus blood samples during ACh testing (in search of microvascular spasm)[19,20] or performing an ACh re-challenge after documentation of spasm and injection of nitroglycerine to assess the protective effect of nitroglycerine. The latter aspects will be covered in other contributions of this JOVE methods collection.

When discussing critical steps in the protocol presented here the first aspect is the vasodilatory effect of nitroglycerin. As coronary angiography is often performed via the radial artery some medication is usually given to prevent radial artery spasm (e. g., nitroglycerin/verapamil). This may have an impact on subsequent vasomotor testing as studies have shown that nitroglycerine

may have an effect on epicardial tone for up to 15-20 minutes[21]. However, a study comparing the effects of any radial artery spasm prophylaxis on ACh testing has not been published so far. In this context it is also debatable when to perform ACh testing (i. e., before or after FFR/CFR/HMR testing). If ACh testing is performed after FFR/CFR/HMR testing, then the vasodilatory effects of nitroglycerine may still be present and influence the results of ACh testing[14]. This is why it is recommended to perform ACh testing before FFR/CFR/HMR testing. However, there has been no direct comparison of these two protocols yet.

Another critical step in the protocol is the use and the positioning of the Doppler flow-/pressure-wire. To avoid any intravascular complications the wire should be placed with caution and ideally in the proximal-mid part of the vessel. For an application in patients with intermediate stenoses especially in the distal portion of the vessel placement with a microcatheter may be advisable. Although the Doppler flow-/pressure-wire has the advantage that a direct Doppler-signal can be heard and seen on the screen obtaining a good signal may sometimes be challenging. A combination of turning and pulling the wire as well as fine tuning with the remote control (e.g., adjustment of scale factor, curve detection and wall filter) solves the problem in most cases.

One important limitation of the method lies in the fact that only the LCA is tested with this protocol. The reason for testing the LCA as the default artery is that two vessels can be challenged at the same time. Nevertheless, in the rare cases in whom the IDP reveals no abnormality in the LCA the RCA should be assessed. Another limitation is that the assessment of microvascular resistance is a rather novel approach and, thus, optimal cut-off values in patients with unobstructed coronary arteries are still a matter of debate. Depending on the method used, either the index of microvascular resistance (IMR; thermodilution method) or the HMR (Doppler technique) is provided. Currently used cut-off values for the diagnosis of microvascular dysfunction are > 25 for IMR[22] and > 1.9[17] or > 2.4[7] for HMR.

The IDP as presented in this article represents one of the most comprehensive forms of coronary vasomotor testing. A major advantage in comparison to non-invasive testing protocols lies in the fact that non-invasive protocols usually are not able to assess coronary spasm. Although it has been suggested to be feasible in a recent publication from Korea[23] there is still a lot of scepticism regarding patient safety as multivessel spasm during non-invasive ergonovine testing may not be adequately controlled. It can be expected that future randomized clinical trials continue to demonstrate the usefulness of the IDP in conjunction with stratified medical therapy. Moreover, the IDP represents the perfect platform for evaluation of new pharmacological agents for treatment of the different endotypes of coronary vasomotor disorders.

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DISCLOSURES:
The authors declare that they have no conflict of interest.

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Figure 1

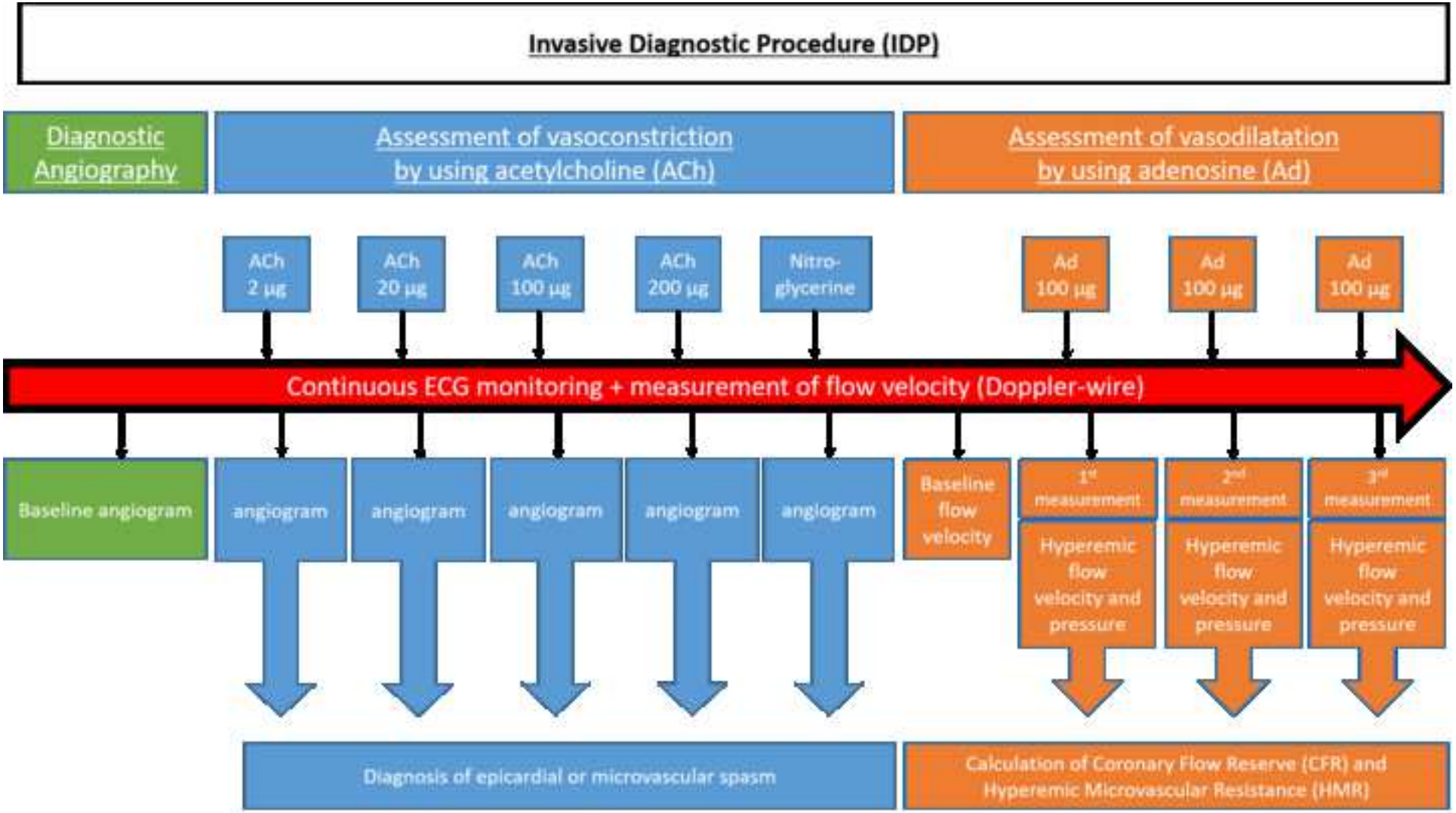


Figure 2

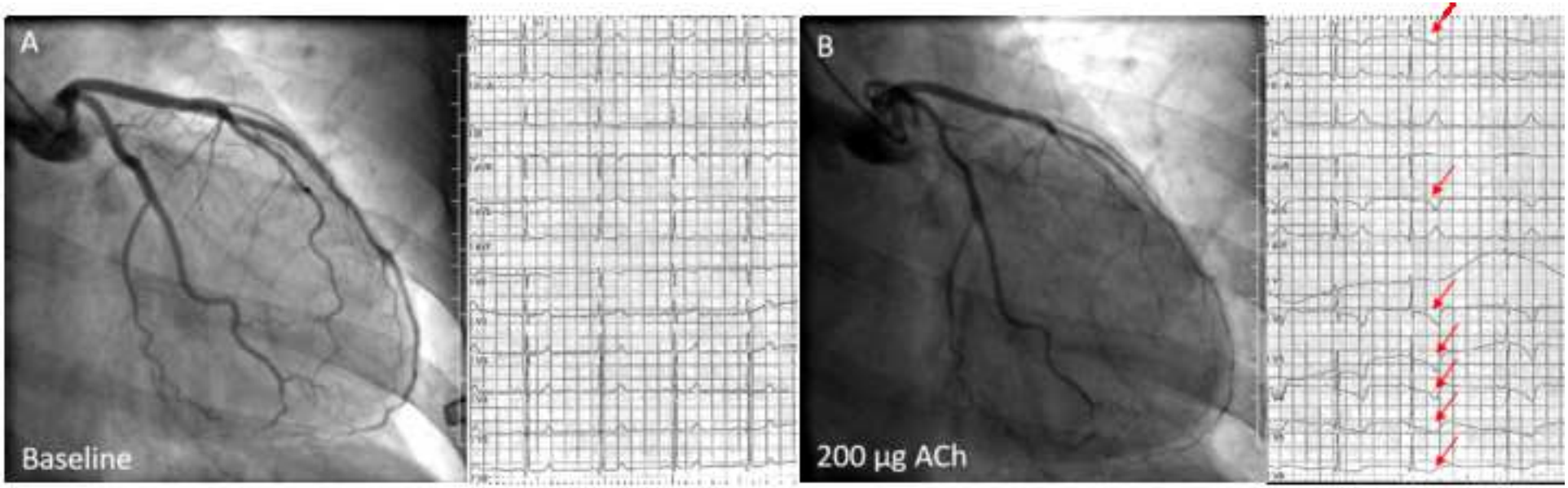


Figure 3

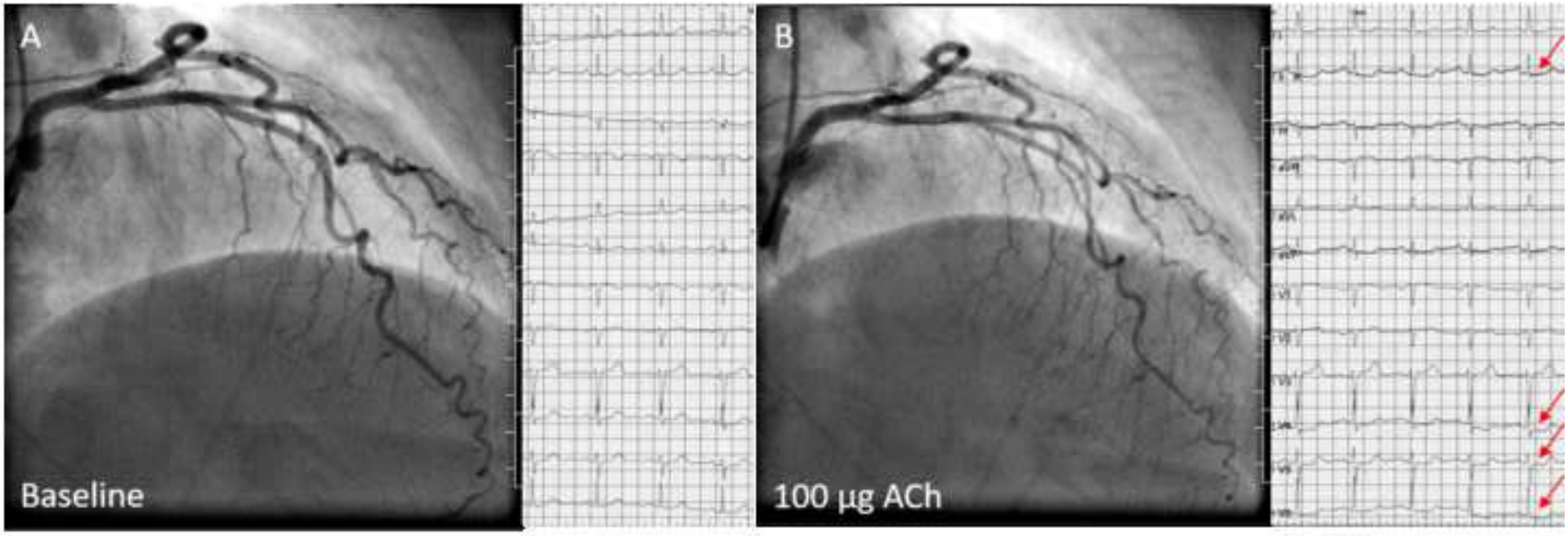
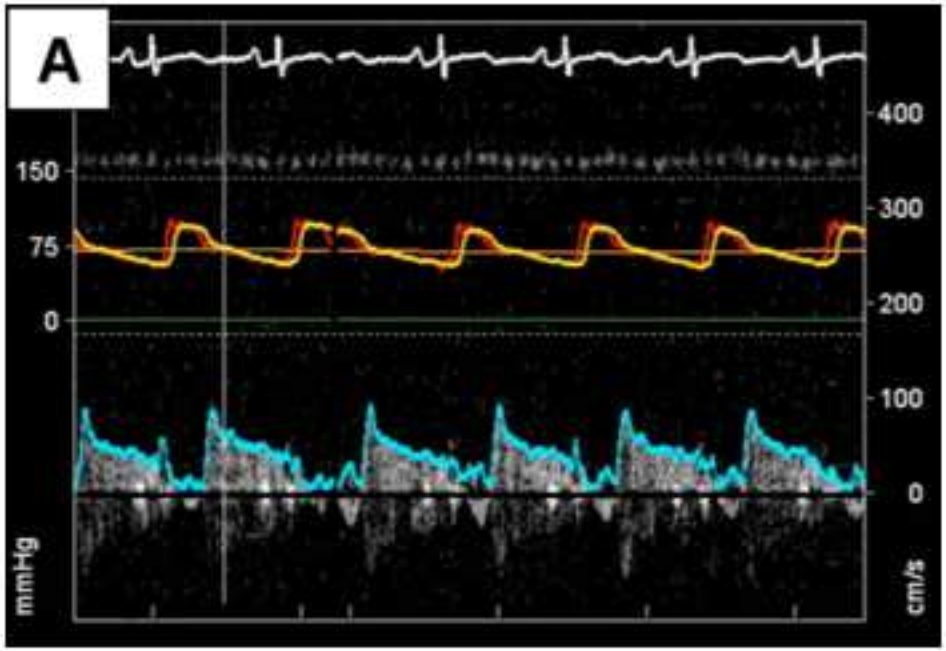
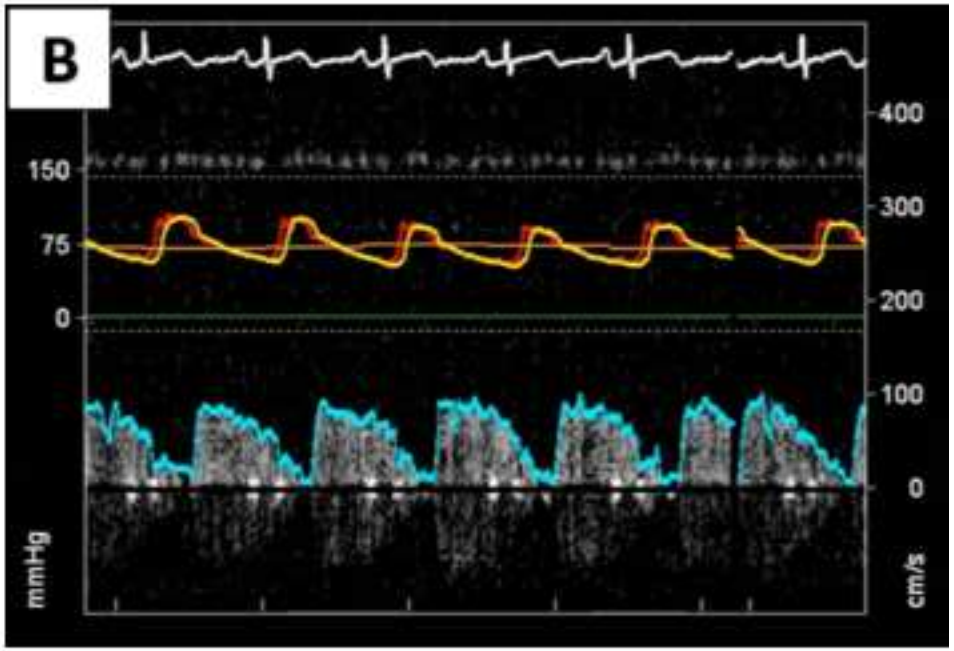


Figure 4



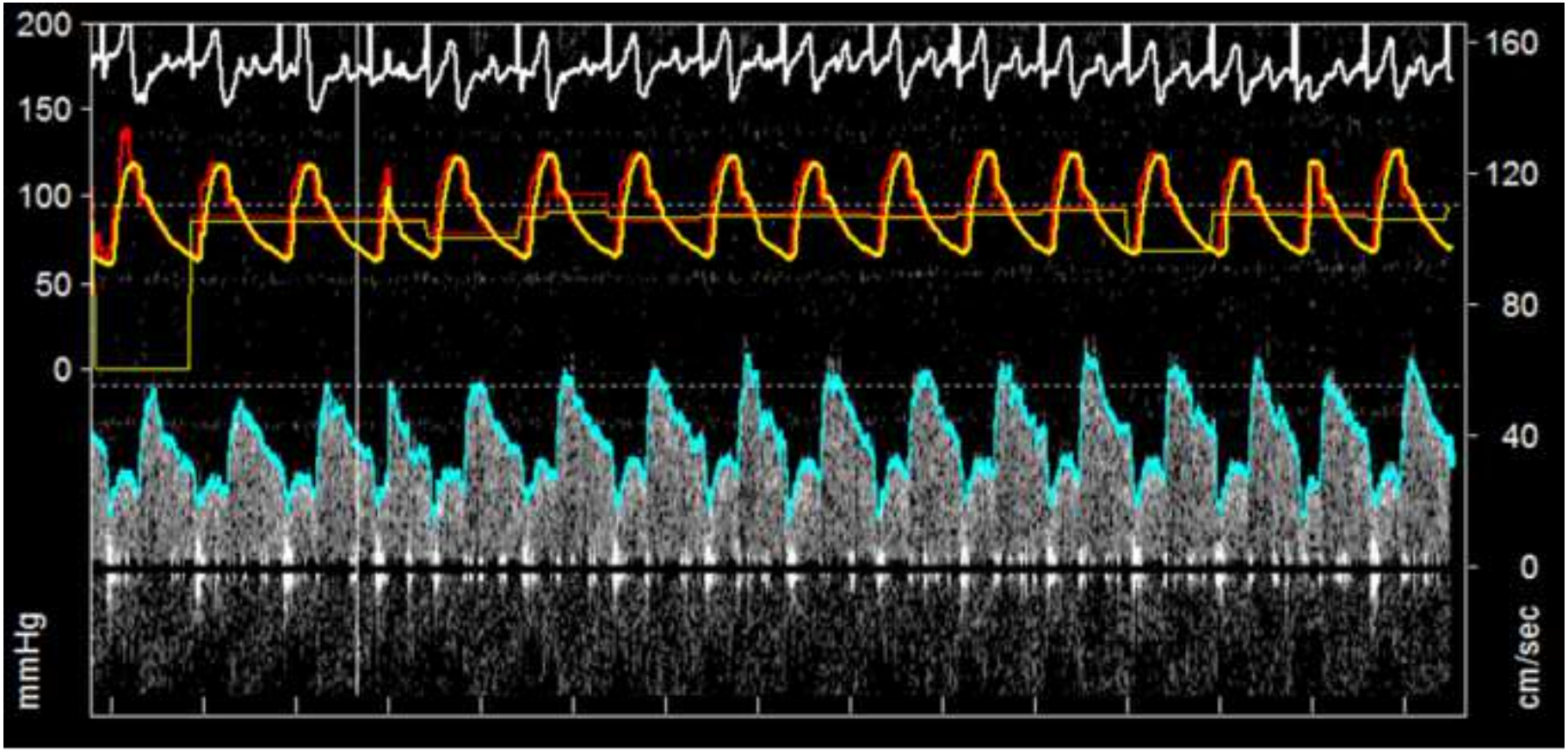
At rest: APV = 36 cm / s



After adenosine: APV = 55 cm / s

$$CFR = \frac{\text{APV after adenosine}}{\text{APV at rest}} = 1.5$$

Figure 5



After adenosine: **APV= 38 cm/s**

P_d = 88 mmHg

$$HMR = \frac{P_d \text{ after adenosine}}{\text{APV after adenosine}} = 2.3$$

1. Diagnostic Coronary Angiography

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Cannula 0,95 x 50 mm (arterial punction)	BBraun	4206096	
Cannula 23 G 0,6 x 25 mm (local anesthesia)	BBraun	4670025S-01	
Coronary angiography suite (AXIOM Artis MP eco)	Siemens	n/a	
Contrast agent Imeron 350 with a 10 mL syringe for contrast injection	Bracco Imaging	30699.04.00	
Diagnostic catheter (various manufacturers)	e.g. Medtronic	DXT5JR40	
Glidesheath Slender 6 Fr	Terumo	RM*RS6J10PQ	
Heparin 5,000 IU (25,000 IU / 5 mL)	BBraun	1708.00.00	
Mepivacaine 10 mg/mL	PUREN Pharma	11356266	
Sodium chloride solution 0.9 % (1 x 100 mL)	BBraun	32000950	
Syringe 2 mL (1x) (local anesthesia)	BBraun	4606027V	
Syringe 10 mL (1x) (Heparin)	BBraun	4606108V	

2. Testing of vasoconstriction using acetylcholine

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Acetylcholine chloride (vial of 20 mg acetylcholine chloride powder and 1 ampoule of 2 mL diluent)	Bausch & Lomb	NDC 240208-539-20	
Cannula 20 G 70 mm (2x)	BBraun	4665791	
Glyceryle Trinitrate 1 mg/mL (5 mL)	Pohl-Boskamp	07242798	
Sodium chloride solution 0.9 % (3 x 100 mL)	BBraun	32000950	
Syringe 2 mL (1x)	BBraun	4606027V	
Syringe 5 mL (5x)	BBraun	4606051V	
Syringe 10 mL (1x)	BBraun	4606108V	
Syringe 50 mL (3x)	BBraun	4187903	

3. Testing of vasodilatation using adenosine

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Adenosine 6 mg/2 mL	Sanofi-Aventis	30124.00.00	
ComboMap Pressure/Flow System	Volcano	Model No. 6800 (Powers Up)	
Pressure/Flow Guide Wire	Volcano	9515	
Sodium chloride solution 0.9 % (1 x 100 mL)	BBraun	32000950	
Syringe 10 mL (3x)	BBraun	4606108V	

RESPONSE TO EDITORIAL COMMENTS **Jan-4th-2021**

Ms. No. JoVE62134
"The interventional diagnostic procedure for assessment of coronary vasomotor disorders: acetylcholine testing followed by adenosine testing"

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

We have thoroughly proofread the manuscript and corrected any spelling or grammar issues. All abbreviations are defined at first use.

2. Please revise the following lines to avoid overlap with previously published work: 97-103, 105-116, 144-147, 156 (Perform…)-162, 167 (Perform…)-177

As suggested, the above-mentioned paragraphs have been revised to avoid overlap.

3. Please shorten your title to "Testing Acetylcholine Followed by Adenosine for Interventional Diagnosis of Coronary Vasomotor Disorders"

The title has been shortened as suggested.

4. 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 and Reagents. For example: ComboWire

All commercial terms have been replaced.

5. Please revise the text, especially in the protocol, to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Sentences with personal pronouns have been revised.

6. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. 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. Please add more specific details (e.g., button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

Further details regarding system settings and button clicks have been added. The protocol steps are now described in sufficient detail to replicate the protocol.

7. After including a one line space between each protocol step, highlight up to 3 pages of protocol text for inclusion in the protocol section of the video. This will clarify what needs to be filmed.

The protocol steps which we would like to be filmed have been highlighted.

RESPONSE TO REVIEWER #1 **Jan-4th-2021**

Ms. No. JoVE62134

"The interventional diagnostic procedure for assessment of coronary vasomotor disorders: acetylcholine testing followed by adenosine testing"

This is a comprehensive description of coronary function testing by a world-leading group with ample experience for many years. It can serve as a global standard for the performance of such measurements. The performance of coronary function testing (CFT) is expected to rise sharply after publication of the CorMica trial and the ESC guideline recommendation to perform CFT in patients suspected for microvascular angina.

We appreciate the reviewer's positive feedback and the constructive comments.

I would replace "interventional diagnostic procedure" by "invasive diagnostic procedure" since no actual intervention is performed.

We thank the reviewer for this suggestion. We agree that no actual intervention is performed during an IDP. Therefore, we have replaced "interventional" by "invasive" as suggested.

In most labs, thermodilution is used instead of doppler-flow. Both methods have their own advantages and disadvantages, however most labs use thermodilution at present. It would be helpful to add also the thermodilution protocol to the current protocol so that it is a more general applicable protocol.

We fully agree with the reviewer that thermodilution is more commonly used compared to Doppler-flow. However, the current protocol includes continuous monitoring of coronary blood flow during acetylcholine testing, which is an advantage of the Doppler-flow technique. Since protocols from other expert coronary physiology research groups that will also be featured in this JOVE spotlight issue will focus on the thermodilution instead of the Doppler-flow method, we would prefer to not add thermodilution to the present protocol.

You use the 20 sec infusion protocol, please explain the rationale for this, also as compared to the much less aggressive 3 minute approach

We thank the reviewer for this important question. Indeed, at our centre we had used the 3 minute approach until about 7 years ago. Inspired by studies from Japan we changed our protocol to the 20 second manual injection, which substantially improved feasibility in daily clinical practice. Notably, previous works by Sueda et al. have shown higher diagnostic yield of the 20 s protocol compared to the 3 min protocol. Moreover, we and others have demonstrated safety of the 20 s approach.

3.3 What should you do in case of small radial artery and spasm? Only verapamil, other short-acting vasodilatory agents?

We thank the reviewer for this question. In fact, so far there is no data on the impact of radial artery spasm prophylaxis on coronary physiology measurements. However, it is conceivable that radial anti-vasospastic treatment may have an impact on the accuracy of acetylcholine spasm testing. Thus, we recommend to omit spasm prophylaxis whenever possible. In patients with small radial arteries we use a 4F catheter without spasm prophylaxis.

However, if radial spasm occurs we give nitroglycerine into the radial artery. If this does not resolve the spasm, we suggest conversion to a femoral access.

3.4 "NOTE: The contrast agent contains Iomeprol, Trometamol, hydrochloric acid and water". Why is this important?

This information has been removed as suggested.

4.4 "Give 5000 IU of heparin intracoronarily (i.c.)" Why intracoronary?

We thank the reviewer for this comment. Of course, heparin can also be administered intravenously instead of intracoronarily. We have changed this accordingly.

5.2 Add also "ask patient for recognizable symptoms of chest pain"

We thank the reviewer for this very important point, which has been added to the protocol.

RESPONSE TO REVIEWER #2 **Jan-4th-2021**

Ms. No. JoVE62134

"The interventional diagnostic procedure for assessment of coronary vasomotor disorders: acetylcholine testing followed by adenosine testing"

Manuscript Summary: The manuscript is well written and the protocol is correct.

Major Concerns: NO major comment

We thank the reviewer for the positive feedback.