Coronary Functional Tests in the Catheterization Laboratory
– Pathophysiological and Clinical Relevance –

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Coronary angiography has long been the only diagnostic tool for the invasive assessment of coronary artery disease. Yet it does not allow establishing the functional severity of epicardial stenoses or vasomotor disorders of the epicardial arteries or coronary microcirculation. Functional tests in the catheterization laboratory have recently emerged as an important adjunct to coronary angiography for providing a comprehensive evaluation of the coronary circulation. In this review, we will describe and interpret the key functional tests used in current clinical practice in different clinical settings. (*Circ J* 2015; 79: 676–684)

**Key Words:** Coronary microvascular disease; Endothelial dysfunction; Epicardial coronary arteries; Functional assessment

Functional tests based on the use of several vasoactive stimuli (Table) and on the measurement of several hemodynamic indexes have recently emerged as standard diagnostic modalities in the contemporary armamentarium of the interventional cardiologist during cardiac catheterization of patients with suspected coronary artery disease (CAD). The functional assessment of coronary circulation is important because it affects the management of patients with CAD. This field has recently evolved thanks also to the substantial advances over the past few years in our knowledge of coronary vasomotion abnormalities. In this review, we will describe and interpret the most important functional tests used in different clinical settings (Figure 1).

**Functional Tests for the Assessment of Endothelial Dysfunction in Asymptomatic Subjects**

Classically, endothelial dysfunction represents the first step of atherosclerosis. The functional approach to evaluating endothelial dysfunction consists in the assessment of coronary vasomotion in response to endothelium-dependent vasodilators such as acetylcholine (ACh), bradykinin, serotonin or substance P or in response to nitric oxide synthase inhibitors.

Briefly, a Doppler-tipped guide wire is placed in the proximal segment of a coronary artery, through a guiding catheter, and Doppler coronary blood flow velocity is continuously recorded. Volumetric coronary blood flow is calculated with the formula validated by Doucette et al. Drugs are selectively infused through the guiding catheter and endothelial function is evaluated by measuring changes in epicardial coronary diameter assessed by quantitative coronary angiography (CAG) and/or of coronary blood flow velocity in response to increasing doses of drug. Most of the information currently available on coronary endothelial dysfunction is based on the utilization of low doses of ACh (usually, 0.182 and 18.2 μg/ml, 2 ml infused over 3 min). Epicardial coronary arteries with an intact endothelium respond to ACh with vasodilation, whereas vessels with endothelial dysfunction show a variable degree of constriction as result of direct activation of muscarinic receptors on vascular smooth muscle cells. Similarly, coronary microvascular endothelial dysfunction is usually defined as an increase in coronary blood flow <50% in response to ACh. Several studies in the past few years have convincingly shown that coronary endothelial dysfunction of epicardial coronary arteries and/or of coronary microcirculation has a worse outcome as compared with patients who have normal endothelial function. It is not well established, however, whether the assessment of coronary endothelial dysfunction improves risk stratification based on the assessment of risk factors. Furthermore, its invasive nature limits clinical applicability. Alternatively, endothelial dysfunction can be investigated in the peripheral circulation by intra-arterial infusion of ACh or by measuring flow-mediated dilation of the brachial artery by use of high-resolution ultrasound during reactive hyperemia. Although the latter has the advantage of being noninvasive, the degree of correlation between endothelial dysfunction in the peripheral and coronary circulations is not well established.

**Functional Tests in Patients With Stable CAD**

**Intermediate Stenoses**

The benefit of percutaneous coronary intervention (PCI) as an initial treatment strategy in patients with stable CAD is still a much debated issue in interventional cardiology. Indeed, the efficacy of myocardial revascularization strictly depends on the extent and severity of myocardial ischemia. Coronary...
mediate stenosis

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tially proposed for assessing the functional severity of inter-

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distal coronary peak flow velocity during maximal hyperemia

flow velocity reserve (CFVR), defined as the ratio of mean
distal coronary peak flow velocity during maximal hyperemia
(usually obtained by intracoronary or intravenous adenosine
administration) to mean peak flow velocity at rest, was ini-
tially proposed for assessing the functional severity of inter-

distal coronary peak flow velocity during maximal hyperemia

Moreover, a recent meta-analysis by Johnson et al

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larization in patients presenting with multivessel disease,

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microvascular dysfunction.

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the relative contribution of epicardial stenoses and coronary

Vasocostrictor

Ergonovine

2–60 μg i.c.
50–300 μg i.v.
Serotonin receptor activation
30 min
Hypertension during i.v. adminis-

Table. Drugs Used for the Assessment of Coronary Vasomotor Function in the Catheterization Laboratory

<table>
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<th>Endothelial-independent vasodilators</th>
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<tr>
<td>Adenosine</td>
<td>60–600 μg i.c.</td>
<td>A2 receptors stimulation</td>
<td>10 s</td>
<td>Bradycardia, bronchoconstriction</td>
</tr>
<tr>
<td></td>
<td>140 μg·kg⁻¹·min⁻¹ i.v.</td>
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<tr>
<td>Papaverine</td>
<td>8–20 mg i.c.</td>
<td>PDE inhibition (less adenosine degradation)</td>
<td>2 h</td>
<td>Ventricular arrhythmias, hepatic toxicity, somnolence-vertigo</td>
</tr>
<tr>
<td>Nitrates</td>
<td>200 μg i.c.</td>
<td>Vasodilation of smooth muscle cells</td>
<td>2 min</td>
<td>Headache, hypotension</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>0.3–0.9 μg/kg i.c.</td>
<td>NO release</td>
<td>2 min</td>
<td>Headache, hypotension, cyanide toxicity</td>
</tr>
</tbody>
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Endothelial-dependent vasodilators:

| ACh* | 0.364–200 μg i.c. over 2–3 min | Muscarinic receptor activation, NO release | 2 min | Bradycardia, hypotension bronchoconstriction |
| Substance P** | 0.7–22.4 pmol/min i.c. | NK1 receptor activation, NO release | 1.5 min | Bronchoconstriction, hypotension |
| Bradykinin** | 0.2–2 μg/min i.c. | B1 and b2 receptor activation, NO release | 2 min | Hypotension, angioedema, cough |

Vasocostrictor:

| Ergonovine | 2–60 μg i.c. | 50–300 μg i.v. | Serotonin receptor activation | 30 min | Hypertension during i.v. administra-

*Dose range Mechanisms of action Approximate half-life Side effects

**Approximate half-life

side effects

Vasocostrictor effect on smooth muscle cells. **No direct vasocostrictor effect on smooth muscle cells. ACh, acetylcholine; i.c., intracoronary; i.v., intravenous; NO, nitric oxide; PDE, phosphodiesterase.

Current Coronary Functional Tests

Landmark studies have introduced FFR in the clinical prac-
tice. The FAME study has convincingly shown a clinical
benefit of FFR-guided vs. CAG-guided myocardial revas-
cularization in patients presenting with multivessel disease,
using a cutoff of 0.80,

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and the FAME 2 study has recently
demonstrated that, in patients with stable CAD, FFR-guided
PCI as compared with medical therapy alone, improves
the outcome principally by a lower rate of urgent revascular-
ization in the PCI group (4.0% vs. 16.3%; hazard ratio (HR): 0.23; 95% confidence interval (CI): 0.14 to 0.38; P<0.001). Moreover, a recent meta-analysis by Johnson et al

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including 9,173 studies with 6,961 lesions showed a continuous and

independent relationship of FFR values with subsequent out-
comes, suggesting that as for every parameter used in deci-
dion-making, cutoff points should be evaluated together with
global risk assessment. Interestingly, they also pointed out that
the measurement of FFR immediately after stenting also
exhibited an inverse relation with prognosis (HR: 0.86; 95%
CI: 0.80 to 0.93; P<0.001), likely from both residual diffuse
disease and imperfect stent deployment, which may prime future
events.

Recently, the NASCET study proposed a useful algorithm based on sequential utilization of contrast medium (which can be utilized more quickly), i.c. and i.v. adenosine (which is the gold standard, but more time-con-
suming) to obtained maximal coronary vasodilation.

The use of this algorithm in daily practice could help in
achieving a physiology-based approach to treatment of coro-
nary artery stenosis, limiting the use of i.c. or i.v. adenosine
for obtaining FFR to equivocal cases only.

Of note, in order to simplify the use of functional tests for
the assessment of intermediate stenoses, new adenosine-free
indexes are emerging. For instance, the instantaneous wave-
free ratio (iFR) is a recently proposed index of stenosis sever-
ity, which uses a translesional pressure ratio as a measure of
functional stenosis severity. It is not based on the use of
vasodilators, but rather on measurement of i.c. pressure during
the diastolic “wave-free” period, a period in the cardiac cycle
when intratube microvascular resistance is inherently stable
and minimized. This wave-free window provides a phase in
which microvascular resistance is significantly lower than that
over the whole cardiac cycle, and coronary hemodynamics are
most suited for assessment of the hemodynamic effects of a
stenosis.

It can be calculated using conventional pressure guidewires
and differs fundamentally from FFR because it does not
require vasodilators such as adenosine for its calculation.

Recently, the ADVICE trial assessed the role of iFR measure-
ment in patients undergoing invasive functional assessment of
Intermediate coronary stenoses (Figure 2). For a FFR cutoff of 0.80, a predefined iFR value of 0.90 provided the optimal cutoff, with a classification match of 80%; when the originally validated 0.75 ischemic FFR cutoff was used as a reference comparison, the agreement between iFR and FFR increased to 88% with the optimal iFR cutoff being 0.85; when accounting for the known 0.75–0.80 FFR gray zone, the classification match between iFR and FFR increased to 93%. Finally, confirming previous reports, a hybrid decision-making strategy with iFR and FFR could avoid the use of vasodilator in 61% of patients, while maintaining 94% overall agreement with FFR classification of lesions. Of note, the impairment of myocardial perfusion is not only determined by the extent of epicardial narrowing, but also by alterations in the coronary microvasculature.

In this context, combined assessment of the coronary circulation with i.c. pressure and flow velocity measurements, as hyperemic microvascular resistance (HMR) and hyperemic stenosis resistance (HSR), allows differentiation of the individual contribution of stenosis and microvascular resistance to coronary flow impediment, thus providing important insights into the role of HMR in the identification of ischemia-inducing coronary stenoses by FFR. They observed that the diagnostic accuracy for inducible ischemia on myocardial perfusion scans of a positive compared with a negative FFR was significantly higher only in the presence of a high HMR (at the 0.75 and 0.80 FFR cutoff). Notably, distal to coro-
Interaction between epicardial and microvascular disease in the development of inducible ischemia that is not underscored by coronary pressure measurements only. Indeed, FFR is influenced by the magnitude of flow through the stenosis; with increasing coronary flow through a stenosis, distal coronary stenoses deemed significant by FFR, the prevalence of inducible ischemia was significantly higher when HMR was high, even though FFR did not differ between HMR groups. Furthermore, the increase in HSR mirrored the increase in HMR and myocardial ischemia. Those findings suggest an interaction between epicardial and microvascular disease in the development of inducible ischemia that is not underscored by coronary pressure measurements only. Indeed, FFR is influenced by the magnitude of flow through the stenosis; with increasing coronary flow through a stenosis, distal coronary

\[ \text{CFVR} = \frac{V \text{ during hyperemia}}{V \text{ during basal conditions}} \]
\[ \text{FFR} = \frac{P_d}{P_a} \text{ during hyperemia} \]
\[ \text{iFR} = \frac{P_d}{P_a} \text{ during "wave free period"} \]
\[ \text{IMR} = \frac{P_d}{\text{MTT}} \]
\[ \text{HSR} = \frac{(P_a - P_d) V \text{ during hyperemia}}{V \text{ during hyperemia}} \]
\[ \text{HMR} = \frac{P_d}{V \text{ during hyperemia}} \]
\[ \text{BSR} = \frac{(P_a - P_d) V \text{ during basal conditions}}{V \text{ during basal conditions}} \]
pressure and, thus, FFR decreases. A low HMR implies that the impediment to coronary flow is relatively low, which may dictate a low FFR value despite the fact that the resistance to flow induced by the stenosis and microvasculature is low at maximal vasodilatation. With increasing HMR, flow through this fixed stenosis will decrease and FFR will increase, despite no alteration in resistance to flow resulting from the epicardial stenosis. However, there is no clinical cutoff value or normal range for HMR. Furthermore, it is debatable whether HMR should be corrected for the contribution of collateral flow to total myocardial blood flow, because its neglect may lead to an overestimation of true microvascular resistance by HMR, although the collateral flow contribution is known to be negligible in the setting of stable CAD of intermediate severity.

The stenosis resistance index during baseline conditions (i.e., baseline stenosis resistance index [BSR]) has more recently been introduced, a notion based on the limited influence of hyperemia on HSR. This index is defined as the ratio of the pressure gradient across the stenosis to the distal flow velocity during baseline conditions (Figure 2) and has been shown to provide a diagnostic accuracy for inducible microvascular ischemia on noninvasive stress testing equivalent to that of FFR or CFVR. However, BSR is in need of further validation before its clinical adoption may be advocated.

In conclusion, the measurement of BSR, HSR and HMR provides more detailed information on the specific contribution of epicardial and microvascular disease to myocardial ischemia and these indexes are more predictive of myocardial ischemia on perfusion scans than FFR; yet, the clinical relevance of these indexes is limited by the lack of prospective studies exploring the effect of their measurement on outcome, which, in contrast, has well been defined for FFR.

Finally, in this clinical scenario, intravascular ultrasound (IVUS) and optical coherence tomography (OCT) are also relied on as alternative options, offering morphological non-functional insights. Yet again, there is not a single morphological parameter (by IVUS or OCT) that has hitherto been validated for deferring coronary revascularization.

### Diffuse Disease

Although studies have documented the high diagnostic efficiency of physiological assessment in minimizing the number of vessels requiring treatment, it is widely recognized that interrogation of an individual stenosis in the presence of tandem lesions or diffuse disease under hyperemic conditions makes PCI planning complex and less practical. These difficulties are related to the hemodynamic interdependence of stenoses under conditions of hyperemia; indeed, hyperemic flow through 1 stenosis is limited by the presence of the other stenoses. Yet treating functionally relevant stenoses while deferring treatment of the nonfunctionally relevant stenoses might improve the outcome. In particular, this may be valuable when some lesions are considered to have higher procedural risk than others.

Another important issue is establishing whether in an anginal patient the myocardial ischemia is caused by moderate but diffuse disease responsible for a progressive pressure loss along an epicardial coronary branch or rather by coronary microvascular dysfunction; it is obvious that these 2 conditions need different treatments.

FFR measured during pullback of the pressure wire under maximal hyperemia can be helpful in the identification of functionally significant stenoses. Indeed, Kim et al found that FFR assessment during pullback pressure is safe and might help in determining target lesions for revascularization by PCI. In vessels with an FFR < 0.8, they stented first the stenosis that caused the largest pressure step-up. In total, 116 stents were implanted and revascularization was deferred in 61.1% (182 of 298) of lesions. There were no events related to deferred lesions.

Another possible approach is a stenosis severity assessment under resting conditions. Recently, Nijjer et al found that iFR measurements during continuous resting pressure wire pullback can provide a physiological map of the entire epicardial coronary branch and can predict the hemodynamic consequences of stenting specific stenoses before PCI, thereby facilitating the intervention and stenting strategy.

However, it has to be taken into account that, in diffuse atherosclerotic disease, coronary microvascular dysfunction also might influence the FFR values. In this context, the index of microvascular resistance (IMR), a pressure-/temperature-tipped guidewire-based quantitative measure of coronary microvasculature function, can be a useful tool for resolving doubtful cases. It is calculated by multiplying the distal coronary pressure by the mean transit time of a 3-ml bolus of saline at room temperature during coronary hyperemia induced by i.v. adenosine and it has been widely showed to be repeat-
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Angina; (2) documented stress-induced myocardial ischemia; (3) absence of obstructive atherosclerotic CAD; (4) absence of organic nonatherosclerotic causes of epicardial CAD (including coronary aneurysms, bridging and anomalies); (5) absence of vasospastic angina (VSA; no epicardial vasospasm with ergonovine or ACh test); and (6) active demonstration of coronary microvascular dysfunction (positive ACh and/or adenosine test results).

In patients in whom the differential diagnosis between microvascular and VSA is uncertain, based on noninvasive testing, it is worth performing an ACh test. The most frequently used protocol to this end is the i.c. administration of incremental doses of ACh (20, 50 and 100 µg into the left coronary artery and 20 and 50 µg into the right coronary artery) over a period of 3 min each. A positive response for MVA is characterized by the onset of angina and typical ischemic ST-segment changes (usually depression) in the absence of focal epicardial spasm. Of note, a diagnosis of MVA based on ACh testing has been reported in approximately 50% of patients referred for CAG because of effort angina and found to have no obstructive atherosclerosis (Figure 5).

In patients with suspected MVA and absence of myocardial ischemia during ACh testing, it is important to assess CFVR using adenosine. A CFVR <2.5 is usually considered as diagnostic of coronary microvascular dysfunction. Because of some significant variability in healthy individuals, however, a cutoff of 2 might be more specific. Recent data indicate that among patients with MVA, a CFR <2 is associated with a worse outcome as compared with patients with CFR ≥2.

It is still unknown whether an abnormal response to ACh and an abnormal CFVR identify different subsets of patients with MVA.

**Functional Tests in Patients With Acute Coronary Syndrome**

Angina at rest with or without raised cardiac enzymes is usually caused by a dynamic and unexpected destabilization...
of an unstable coronary plaque with superimposed coronary thrombosis.60

However, the introduction of high-resolution imaging modalities such as OCT has allowed the establishing that a subset of patients with rest angina exhibit epicardial stenoses with stable features, thus suggesting an important role for coronary vasomotor dysfunction.61 An extreme form of coronary vasomotor dysfunction in this setting is epicardial or microvascular spasm, which needs to be systematically investigated, particularly in patients with predominantly rest angina who do not exhibit obstructive atherosclerosis on CAG. In a recent study of patients with predominantly rest angina who underwent CAG and were found to have no obstructive lesions on CAG, ACh testing revealed epicardial or microvascular spasm in 49% of patients.62

VSA
Numerous agents have been proposed for spasm provocation testing, including ergonovine, ACh, neuropeptide Y, histamine and dopamine.63-68 However, a large body of evidence supports utilization of ergonovine and ACh for clinical practice. A positive response to ACh (Figure 6)63 or ergonovine is defined as a transient occlusion (>90% narrowing) of a coronary artery branch with signs and symptoms of myocardial ischemia (angina/ST-segment changes).69,70 Various testing protocols using i.c. and i.v. ergonovine administration have been described (Table).70-74 Importantly, Hackett et al demonstrated that induction of coronary artery spasm (CAS) with i.c. ergonovine might be safer than that induced by i.v. administration.64 Furthermore, although i.v. ergonovine provocation testing has good sensitivity (100% with angina as part of the diagnostic criteria, and 94% with ST-segment elevation),71 reports show the frequency of provoked CAS with i.c. ergonovine to be 2.2–2.6-fold higher than with i.v. testing.74 Specificity of i.v. and i.c. ergonovine provocation testing are similarly high at >90%. Despite high sensitivity, a negative test cannot always exclude CAS. A recent observational study70 evaluated 1,244 patients with VSA who underwent different i.c. provocation tests (40% ergonovine, 57% ACh, 2% ergonovine+ACh, 1% other). The overall incidence of arrhythmic complications was 6.8%, which is comparable to 7.0% during spontaneous angina episodes. They reported a 5.5% major adverse cardiovascular event rate during the 32-month follow-up period. After multivariable analysis, mixed (focal and diffuse) multivessel spasm predicted major adverse cardiovascular events (adjusted HR: 2.84; 95% CI 1.43 to 6.03, P<0.01), whereas provocation-related arrhythmias (defined as ventricular tachycardia, ventricular fibrillation, and bradyarrhythmias) did not.70

Unstable MVA
Coronary microvascular spasm is characterized by transient transmural myocardial ischemia, as indicated by ST-segment changes, during spontaneous or provoked angina, in the presence of normal epicardial coronary arteries. It may be considered as the unstable presentation of MVA.75 Approximately 25% of patients with acute coronary syndrome and no obstructive CAD have evidence of microvascular spasm, although an increase in the troponin concentration is infrequent.76 In this context, unstable MVA, similar to what we previously described for stable MVA, can be diagnosed when an i.c. ACh test reproduces the symptoms usually experienced by the patient and triggers ischemic ECG changes, in the absence of focal epicardial spasm (Figure 5).77

Conclusions
Functional tests in the catheterization laboratory are emerging as important tools for epicardial and microvascular assessment. Importantly, their use should be coherently integrated in different clinical settings. Functional tests will probably lead to a more comprehensive assessment of CAD and guide treatment based on a full understanding of the underlying mechanism of disease.

Disclosures
The authors deny any conflict of interest and any financial or other relationship with other people or organizations.
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