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Application of Fractional Flow Reserve in the diagnostics of coronary artery disease

PhD thesis

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1. Table of content

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2. The list of abbreviations

CAD - Coronary artery disease
CFR - Coronary flow reserve
DS - Percent diameter stenosis
FFR - Fractional flow reserve
FFR_{myo} - Myocardial fractional flow reserve
LAD - Left anterior descending coronary artery
LCA - Left coronary artery
LCx - Left circumflex coronary artery
LM - Left main stem
MACE - Major adverse cardiac event
MI - Myocardial infarction
MLD - Minimal luminal diameter
P_a - Aortic pressure
PCI - Percutaneous coronary intervention
P_d - Distal coronary pressure
QCA - Quantitative coronary angiography
RCA - Right coronary artery
RD - Reference diameter

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3. Introduction

3/1 – Background

Despite all our preventive and therapeutic efforts cardiovascular disease is still the leading causes of death globally. In 2012 17.5 million people died in cardiovascular diseases, representing one third of the worldwide human mortality. More than half of these deaths occurred due to coronary artery disease (CAD). Since cardiovascular pathologies are typically the diseases of developed countries, numbers are not better in the European Union either. Latest statistics showed 1.9 million cardiovascular deaths yearly, taken almost 40% of the total mortality. Accordingly the costs are enormous, reaching 196 billion euro a year, where more than half derived from the health care costs, while the rest is coming from the productivity losses and the informal care. Therefore, cardiovascular diseases mean a tremendous medical and economical issue. However, it is important to realize that cardiovascular diseases are mainly well treatable when detected on time, and so cardiovascular death is well preventable with careful and appropriate medical care. Accordingly, as declared by the World Health Organization, "*people with cardiovascular disease or who are at high cardiovascular risk (due to the presence of one or more risk factors such as hypertension, diabetes, hyperlipidemia or already established disease) need early detection and management using counseling and medicines, as appropriate.*" [1]

As a straight consequence, the pressure on the medical system and its players, the hospitals and medical crews, is enormous to manage properly the diagnostic work-up and treatment of patients with suspected CAD. Accordingly, accurate definition of significant or clinically relevant CAD has been in the focus of physiologists for many decades.

As principle the CAD becomes significant, when the blood supply of the myocardium is limited by a coronary stenosis to be less than the demand at *any* condition. However at the early years of invasive cardiology this principle was very poorly definable in the clinical practice, especially because true in vivo physiologic measures on the level of coronary arteries were markedly limited if not impossible at that time. Therefore, there was an urging need to translate physiology measures to morphology parameters, which were already approachable to some extent by coronary angiography. [2]

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Milestone data have been published in the 1970ies by the working group of Gould, who aimed defining a clear link between morphological impairment and hemodynamic consequences based on animal experiments. Extensive measurements were performed in twelve anesthetized dogs after thoracotomy. Coronary blood flow was evaluated in the left circumflex coronary artery using perivascular electromagnetic flowmeter, while different levels of stenoses were generated by a snare, proximally positioned to the flow sensor. Coronary flow was determined during resting conditions, as well as during pharmaceutically induced hyperemia. Flow / stenosis correlation curve was determined

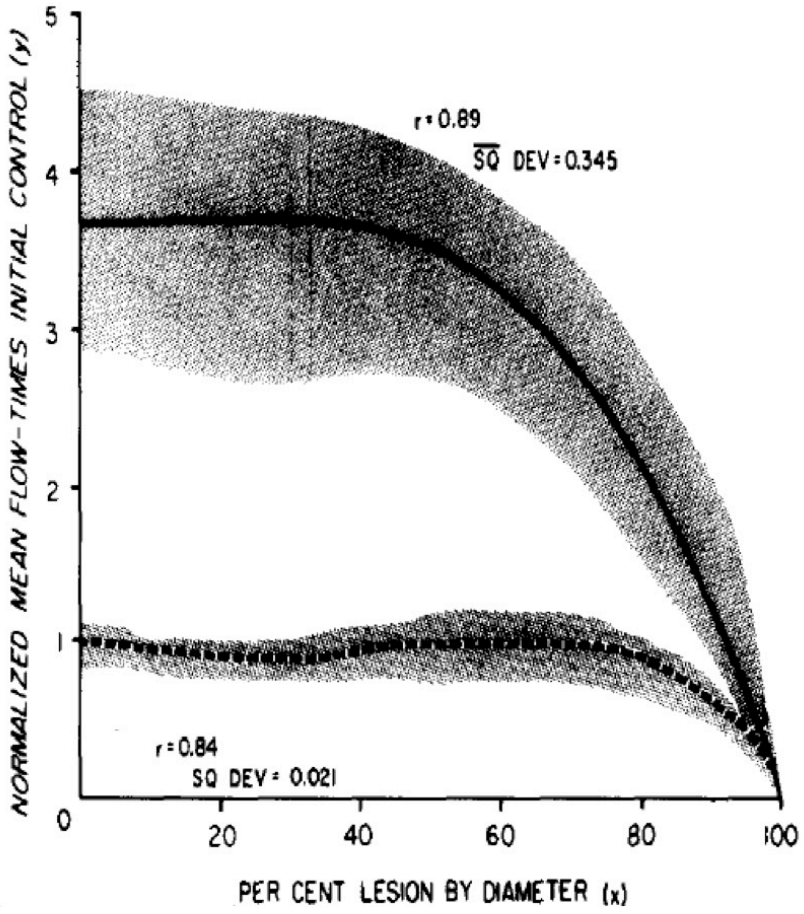

Figure 1 – Coronary flow measurements in dogs, while inducing different level of coronary stenosis. Results show that during resting conditions (dotted line) coronary blood flow remains stable until approximately 85% diameter stenosis, while during hyperemia (solid line) coronary blood flow starts to decline steeply already around 45% diameter stenosis. *Adapted from Gould et al. [4]*

from the sequential measurements. As **Figure 1** shows, due to massive autoregulation mechanisms the resting flow had a stable, uneffected plateau until the constriction has reached 85 percent and it started to decline only above that stenosis severity. However, hyperemic response became damped already when 45 percent coronary narrowing was induced, and decreased precipitously much before resting flow was even affected. [3;4] Physiologic background of this finding can be well explained by the excellent autoregulatory mechanism of the coronary artery system, including a macrovascular compartment and a microvascular compartment. [5-9] Although the description of all the complex humoral and cellular mechanisms behind is beyond the scope of this work, but the principle is crucial to be understood. The autoregulatory mechanisms of the coronary circulation have the role to stabilize the flow within a wide range of physiologic (or even pathologic) hemodynamic conditions, namely between approximately 50 and 140 mmHg mean arterial pressure. This stability can be achieved by the instantaneous constriction or relaxation of the precapillary resistance sphincters, resulting in increase or decrease in the total microvascular resistance, as a compensating response on changes in perfusion pressure. Accordingly, the resting coronary blood flow becomes independent from the hemodynamic conditions, at least within the wide physiologic range. [10] However, when we pharmacologically 'exhaust' the compensating mechanisms by using hyperemia inducing drugs, then markedly higher (uncontrolledly high) blood flow can be observed at baseline, which starts do decline already at stenosis of much slighter severity.

As physiologic measurements in humans were markedly limited due to lack of proper tools and questionable ethical reasons, based on the findings of Gould et al. 50% diameter stenosis (DS) became the cornerstone of defining significant or obstructive coronary artery disease.

Consequently, the 50% diameter stenosis cut-off value has been used universally for 30 years: (1) it was applied for the validation of risk stratification formulas [11-14], to indicate and justify revascularization [13;14], to serve as an endpoint in studies on revascularization strategies [15-22] and to validate novel non-invasive techniques. [23-26]

However, one might realize, while this cut-off value was defined in 'young, healthy dogs' in standardized coronary location, the population who undergoes cardiac

catheterization is more heterogeneous: in terms of age, in terms of extent of coronary sclerosis, in terms of cardiac- and non-cardiac comorbidities or in terms of medical history. Illustratively, it is wishful thinking that a stenosis of 50% DS in the left main stem of a young man has the same hemodynamic relevance, as a stenosis of 50% DS in the second marginal of an old, diabetic lady, with extensive post-infarct scar in the supplied territory.

Aligned with this example, the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial has pointed out first that revascularization is not as omnipotent as one might have believed. The trial has showed that with the indications of that time percutaneous revascularization not provides any benefit in terms of death, myocardial infarction or other major cardiovascular events, as compared to medical therapy. Of course, this finding has raised the question, whether our therapy is too weak or not the patients were selected who are really in need for treatment, i.e. revascularization? [17]

One must realize that the main prognostic factor is not purely the angiographic severity of the CAD, but the true *presence* and *extent* of ischemia. Therefore benefit from revascularization can be only expected when it eliminates ischemic risk, so when the ischemia inducing potential of that given coronary stenosis is proven [27] **Figure 2.** As consequence, there was a growing awareness that the link between the angiographic metrics and the ischemic potential of a stenosis is elusive and the revascularization-guiding power of angiographic metrics alone became doubtful, putting more and more emphasis on the need for additional functional evaluation. [28-32]

The presence, localization and extent of ischemia can be assessed by a large number of non-invasive testing, purportedly with acceptable accuracy. [33] Paradoxically, in clinical practice, only a minority of patients, who undergo a coronary angiography, undergoes also any clinically meaningful non-invasive functional test. [34;35] This disconnect relates mainly to the large number of clinical and logistical conditions that make the test difficult to perform, not uniformly available, time- or cost-consuming or challenging to interpret.

The development of coronary angioplasty [36] has already granted access to intracoronary pressure measurements opening a potential approach to physiologic understanding. In the early era of interventional cardiology a post-angioplasty pressure

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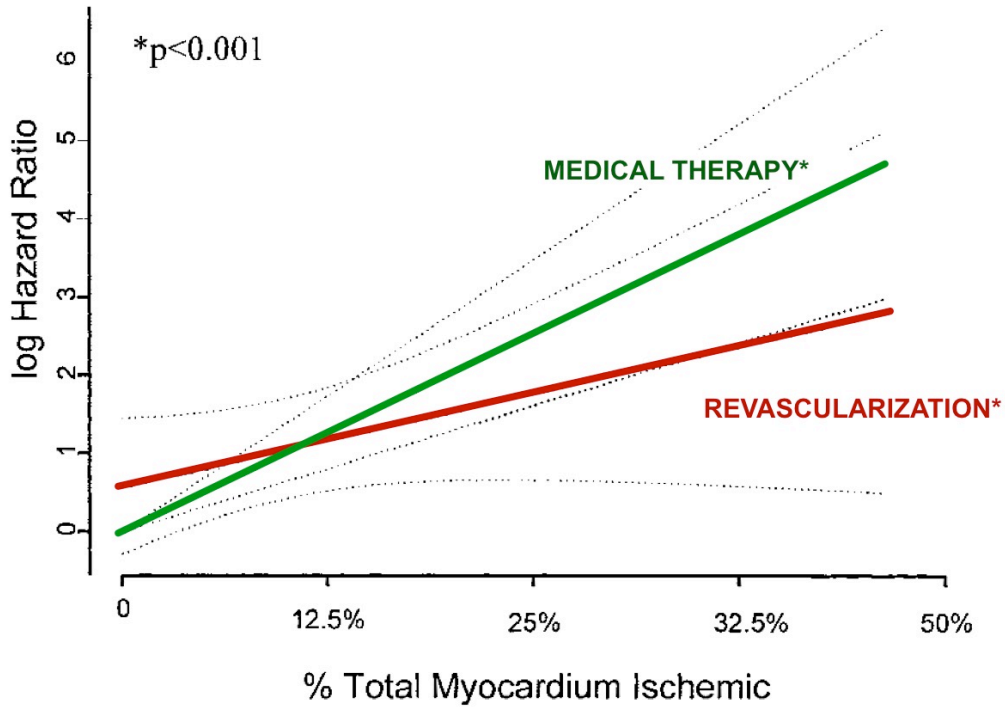

Figure 2 – Clinical outcome after revascularization versus medical therapy, in correlation with the extent of ischemia. Comparison of mortality after revascularization (red line) versus after pure medical therapy (green line) in patients with CAD. Clinical benefit from revascularization as compared to medical therapy alone can be expected only in case of relevant territorial ischemia, namely more than 10% of the total myocardial mass. *Adapted from Hachamovits et al. [27]*

gradient smaller than 20 mmHg was found indicative of a favorable clinical outcome. [37] Also, coronary wedge pressure was a well recognized marker of collateral function. [38-40] But the interest in coronary pressure measurements faded away due to three (primarily technical and conceptual) factors precluding the use of pressure measurement to assess stenosis severity: (1) the space occupied by the balloon in the stenosis induced an unpredictable overestimation of the pressure gradient; (2) the pressure gradient is highly dependent on aortic pressure; (3) resting rather than hyperemic pressure measurements were used.

These limitations have been circumvented by the development of pressure measuring guide wires [41] and the invention of the concept of fractional flow reserve (FFR) by

Pijls and coworkers in 1993 [42-44]. As it will be shown, thanks to all technical and conceptual advantages, after 20 years FFR become the standard of reference to define the ischemic potential of epicardial stenoses of intermediate angiographic severity based on outcomes of randomized trials. Accordingly, most recent revascularization guidelines strongly recommend, when non-invasive evaluation of ischemic burden is missing, invasive functional assessment by FFR has to be applied at the time of invasive coronary angiography in order to take appropriate decision about indicating or deferring revascularization. [45]

3/2 – Fractional flow reserve

3/2.1 Definition

FFR is defined as the ratio of maximal hyperemic myocardial blood flow in the presence of a stenosis to the physiologic maximal hyperemic myocardial blood flow in the same territory but in the absence of any stenosis. [42-44] Accordingly, this index expresses maximal achievable blood flow as a fraction (percent) of its theoretically normal value (i.e. in case the epicardial narrowing was absent). Thus, the value of FFR quantifies to what extent hyperemic flow is reduced by the presence of the epicardial narrowing. As a corollary, FFR also quantifies to what extent a revascularization procedure could increase myocardial perfusion. For example, an FFR value of 0.66 corresponds to maximal myocardial blood flow of 66% of its normal value. In this example, restoring the epicardial conductance will increase maximal flow by approximately 50% as compared to its pathologic status.

3/2.2 Calculation

It has been demonstrated that FFR, a ratio of two flows, can be calculated from the ratio of two pressures [44], the distal coronary pressure (P_d) divided by the proximal pressure, or aortic pressure (P_a) under maximal macro- and microvascular dilation. Since in a strictly normal epicardial artery P_d equals P_a [46], each patient and each segment acts as its own control.

Description of the complete mathematical derivation and its physiologic background is beyond the scope of this work. The summary of the princeps is indicated and explained in **Figure 3**. [42-44] Note, the accuracy and the physiologic meaning of FFR depend on

1. Fractional flow reserve (FFR) expresses the ratio of maximal myocardial flow in the presences of the stenosis (Q_s)and the normal hypothetical maximal myocardial flow of the same territory without the stenosis (Q_n):

$$FFR = \frac{Q_s}{Q_n}$$

2. Since flow can be expressed as the ratio of driving pressure i.e. the difference between aortic pressure (P_a) or distal coronary pressure (P_d) and venous pressure (P_v) to microvascular resistance (R):

$$FFR = \frac{(P_d - P_v) / R}{(P_a - P_v) / R}$$

3. Since the measurements are obtained under conditions of maximal microvascular dilatation, resistances are minimal and equal, and R cancels out:

$$FFR = \frac{(P_d - P_v)}{(P_a - P_v)}$$

4. Since, P_v is negligible as compared to P_a, its influence can be neglected:

$$FFR = \frac{P_d}{P_a}$$

Figure 3 – Basic principle of measurement and calculation of fractional flow reserve.

FFR – fractional flow reserve; $\mathbf{Q_n}$ – maximal flow in normal artery; $\mathbf{Q_s}$ – maximal flow in stenotic artery; $\mathbf{P_v}$ – venous pressure; $\mathbf{P_a}$ – aortic pressure; $\mathbf{P_d}$ – distal coronary pressure; **R** – resistance of the coronary circulatory system

the induction of maximal hyperemia. Only upon abolition of all mechanisms responsible for the autoregulation of resting blood flow, one can state that the ratio of the P_d/P_a equals the corresponding flow ratio. Therefore, during maximal hyperemia and completely blocked autoregulatory mechanisms a linear relationship can be established between perfusion pressure and hyperemic flow, above 40 mmHg perfusion pressure. [42-44;47] Note:

a. FFR can be derived from a ratio of two pressures, but fundamentally corresponds to ratio of two flows. Accordingly, FFR is the only pressure-derived index with a true physiologic meaning.

b. In contrast to a general belief, the definition of FFR does not assume that the microcirculation is normal. FFR quantifies the extent to which the epicardial stenosis contributes to reduced myocardial perfusion, regardless of the status of the microvascular function in that given patient. The microcirculatory function is

presumably often abnormal in patients undergoing coronary angiography and it is difficult to assess quantitatively, but microcirculatory dysfunction is not the target of interventional strategies and revascularization therapy. E.g. in a 80-year-old diabetic patient with an FFR of 0.85 in the proximal right coronary artery, the epicardial stenosis accounts for 15% of the reduction of myocardial perfusion. It can be the case that this patient has myocardial ischemia due to severe microvascular disease, which limits the maximal achievable coronary blood flow, but this cannot be eliminated by stenting a stenosis in the epicardial vessels. It is likely when this patient was 40 year younger and not diabetic and had a completely healthy microvascular compartment, the hyperemic flow would have been markedly larger, and in that status the exact same stenosis would have been a significant burden and was associated with a lower FFR. However, for clinical decision-making about revascularization it is important to know the present status of the patient and not that of 40 years ago.

More importantly, as described above, the formula of FFR calculation neglects central venous pressure, as it is considered to be a magnitude smaller as compared to the arterial pressures and therefore to have minimal or no impact on the calculation. For the 'average' CAD population it might be generalizable true. However as the use of FFR became broader and it is interrogated even in more severe patient population, such as valvular disease or cardiomyopathies, where the filling pressures are usually (far) above the normal range, the applicability of FFR became questioned. Accordingly the concept of FFR is nowadays often criticized due neglecting the right atrial pressure and indicated as a potential limitation in accuracy. [48;49] **Therefore, as specified later, one of the goals of our work was to evaluate whether incorporation of central venous pressure in the FFR formula, namely measurement of myocardial FFR (FFR_{myo}) has any clinical relevance as compared to 'traditional' FFR measurement.**

3/2.3 Practical aspects

- *Pressure measuring guide wires.*

A number of devices are commercially available: the PressureWire™ (St. Jude Medical, St. Paul, Minnesota, USA) and the WaveWire™ (Volcano, San Diego, California, USA), which are 0.014" guidewires, equipped with an electric pressure sensor; the

OptoWire™ (Opsens Medical, Quebec, Canada), which is also a 0.014" guidewire, equipped with a fiberoptic pressure sensor. In addition, a microcatheter with a fiberoptic pressure sensor, called Navvus™ (Acist Medical Systems, Eden Praire, Minnesota, USA), that can be advanced over a regular 0.014" guidewires has been developed to measure distal coronary pressure. The vast majority of the clinical research has been conducted with the PressureWire™ (St. Jude Medical, St. Paul, Minnesota, USA).

In the pressure-sensor guidewires the sensor itself is situated 3 cm proximal to the tip, at the junction between the non-radio-opaque and radio-opaque portions of the wires. The 'maneuverability' of the newest generations of all pressure-measuring guidewires is almost equivalent to any standard workhorse percutaneous coronary intervention (PCI) guidewire. Intravenous or intraarterial heparin should be given before manipulating a wire in the coronary arteries.

- *Hyperemia*

FFR can be accurately obtained only during maximal hyperemia, when all autoregulatory mechanisms are 'switched off'. Intracoronary nitrate must be administered to reach full dilation of the epicardial vessels. Other hyperemic agents, listed in **Table 1**, completely and reproducibly minimize microvascular resistances. [42-44] With the exception of nicorandil and nitroprusside, the effect of most hyperemic agent goes through the release of endogenous adenosine. The most frequently used hyperemic stimuli are intracoronary and intravenous adenosine.

Intracoronary adenosine: Based on early validation experiments the optimal dosage of intracoronary adenosine was defined as 60 µg in the right coronary artery and 100 µg in the left coronary artery. However there are recurrent debates about the potential beneficial effect in terms of increased accuracy, when higher dosages are applied for FFR measurements. [50] ***As specified later, one of the goals of our work was to define clearly the optimal dosage of intracoronary adenosine for the accurate and reliable measurement of FFR.*** An example of a typical coronary pressure tracing during the administration of intracoronary adenosine is shown in **Figure 4**.

Intravenous adenosine: Most early clinical data have been obtained with intravenous adenosine. This method provides with stable maximal hyperemia, but also allows maintaining it for a longer time, when indicated. Therefore, in daily practice it is mainly applied, when (1) intracoronary administration is unreliable (for example in case of

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Table 1 – Different pharmacons used for hyperemic stimulus

Epicardial vasodilatation	**Dosage**
• Isosorbide dinitrate:	200 µg intracoronary bolus
Microvascular vasodilatation	**Dosage**
• Adenosine:	60-100 µg intracoronary bolus
140 µg/kg/min intravenous infusion	
• Papaverine:*	8-12 mg intracoronary bolus
• Nitroprusside:	0.6 µg/kg intracoronary bolus
• Regadenoson:	400 µg intravenous slow bolus

* Not recommended due to frequent occurrence of Torsade De Pointes ventricular tachycardia

ostial stenosis) or when (2) pullback measurement has to be performed for the evaluation of multiple serial stenoses. It is recommended to infuse through a central

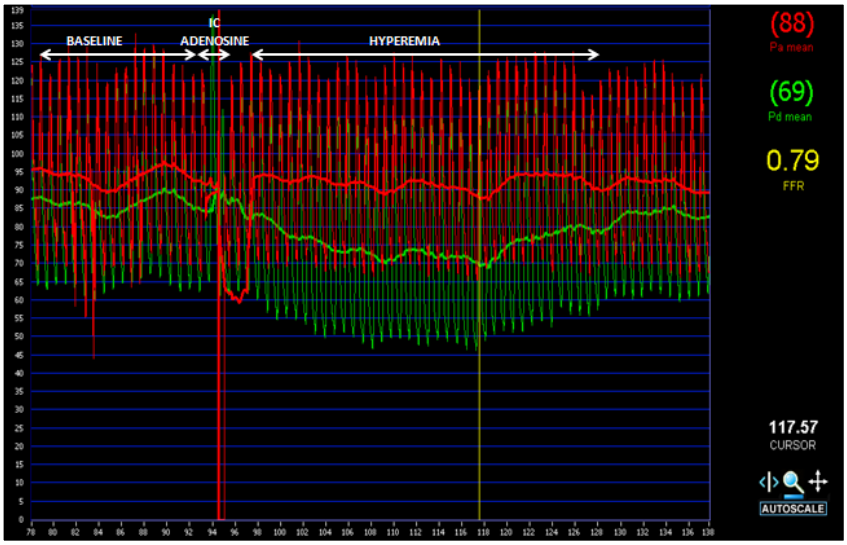

Figure 4 – Fractional flow reserve tracing. An example of a typical coronary pressure tracing during the administration of intracoronary adenosine. *Red* curve indicates the aortic pressure, while *green* curve indicates the distal coronary pressure. Different phases of the measurement, namely baseline, induction of hyperemia and hyperemic phase are indicated on the top of the figure. Please note the separation of the two curves after administration of intracoronary adenosine.

venous line 140 μg / body weight kg / minute adenosine, however data suggests that a simple antero-cubital venous access is also reliable in most patients. The latter has an increasing importance and advantage with the increasing use of radial approach. [51]

3/2.4 Fractional flow reserve characteristics

FFR has a number of characteristics making this index particularly suitable for functional assessment of coronary stenoses and clinical decision-making in the catheterization laboratory.

- *Normal value*

Since in a normal epicardial artery there is virtually no decline in pressure, P_a equals P_d along the whole coronary artery, meaning that the normal value of FFR is 1.0. An unequivocally normal value is easy to refer to. FFR of angiographically normal coronary arteries was investigated, comparing individuals without any atherosclerosis and patients with angiographic stenoses in *another* coronary artery. In the first group FFR was near unity, indicating no resistance to flow in truly normal coronary arteries. In contrast, in the second group of patients with remote coronary atherosclerosis, FFR was found significantly lower indicating the presence of diffuse atherosclerosis without any solid narrowing. Note that angiogram defines stenosis as a narrower segment as compared to the surrounding segments, hypothesizing that the latter are normal. [52]

- *Influence of blood pressure and heart rate*

For a given coronary stenosis FFR has been shown to be stable despite changes in heart rate and blood pressure, at least within the physiologic autoregulatory range. [53;54] Several factors may explain this relative insensitivity to hemodynamic changes: (1) In contrast to any flow velocity based measurement, the pressure signal is devoid of ‘noise’; (2) Aortic and distal coronary pressures are measured simultaneously, and will therefore be influenced simultaneously by systemic pressure and by heart rate; (3) Adenosine agonists induce an extremely reproducible maximal vasodilation of the microvasculature, while nitrate derivates expand the epicardial vessels reliably and reproducible. Accordingly, FFR measurements are highly reproducible. Both, the

clinical value of FFR, as well as the trust in its value for decision-making are based on this reproducibility.

- *Contribution of collaterals*

Oxygen and other nutrients can reach the myocardium antegrade, through the normal epicardial arteries, or retrograde through collateral vessels. Distal coronary pressure takes into account both antegrade and retrograde flows. Therefore, FFR accounts for the summed blood supply, including the flow through native coronary arteries, as well as the collaterals. Collateral function alone can be assessed by distal pressure during occlusion. The higher the distal occlusive wedge pressure, the better the protective role of collaterals for myocardial function. [40;55;56]

In addition, it is important to keep in mind that in case of a coronary artery, which provides collaterals to an occluded artery and therefore supplies much larger myocardial territory then normally, blood flow will increase. Accordingly, in case of a stenosis the transstenotic gradient will be larger and FFR will be lower than if collaterals were absent. **Figure 5**. Yet the influence of collaterals on the donor artery is modest. [57]

- *Spatial resolution*

When the decision has to be taken in the catheterization laboratory, the operator has to know exactly, where the ischemia is generated: in which vessel, in which segment, at which millimeter. Since the location of the pressure-sensor of the guidewire is accurately definable and modifiable within the coronary tree, FFR can provide the operator with an extreme high spatial resolution, exceeding any other functional test.

- *Cut-off values*

In keeping with its theoretical definition, the normal value of FFR equals 1.0. An FFR value of 1.0 indicates the absence of resistance along the epicardial artery. Any value lower than 1.0 is abnormal, but it is not necessarily able to induce myocardial ischemia.

Validation studies have showed that the optimal cut-off threshold for ischemia is between 0.75 and 0.80. [58;59] These cut-off values were defined by comparison to one or several non-invasive tests, which had to be positive prior to revascularization and reverse to negative after revascularization. FFR value >0.80 is associated with the absence of ischemia during stress, while stenoses with an FFR ≤0.80 are almost

uniformly associated with myocardial ischemia and potential clinical consequences. [44;58-60]

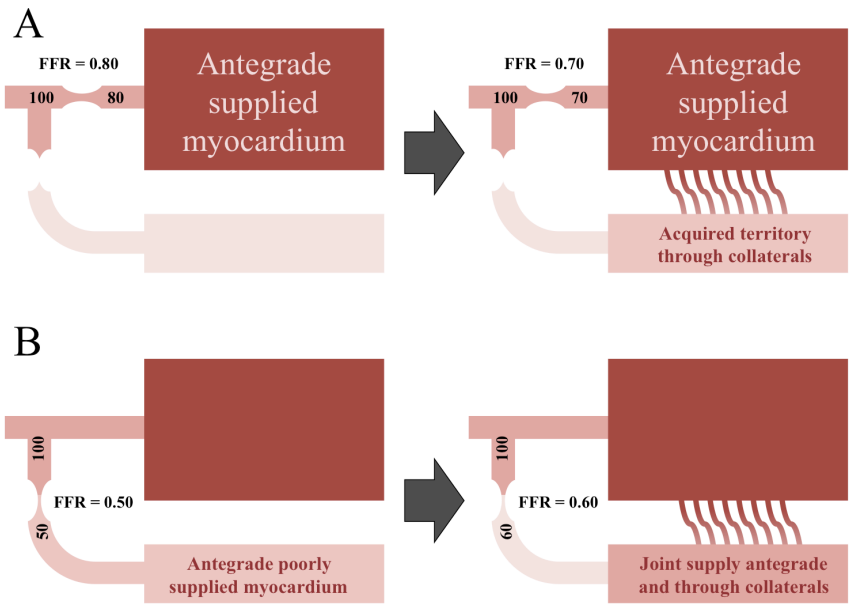

Figure 5 – Role of collaterals. In case of a coronary artery, which provides collaterals to an occluded artery, the supplied myocardial mass and consequently the blood flow are much larger then normally. Accordingly, in case of a stenosis the transstenotic gradient will be larger and FFR will be lower than if collaterals were absent. (**Panel A**). On the contrary, in case of a coronary artery with tight stenosis, whose territory is also supplied through collaterals from another artery, the transstenotic gradient will be smaller and FFR will be higher than if collaterals were absent. (**Panel B**)

3/2.5 Clinical outcome data and specific anatomic settings

Clinical outcome data of patients in whom decision-making has been based on FFR are available in most anatomic settings:

- *Angiographically intermediate stenoses*

The distinction between very mild and critical stenoses is usually not problematic by conventional coronary angiography, but decision-making in the range between can be cumbersome or even inconsequent. [61] Accordingly, stenoses angiographically defined

between 30 and 90% are usually considered ‘moderate’, ‘intermediate’, ‘dubious’, ‘borderline’, ‘non-flow limiting’. The richness of our vocabulary to describe these stenoses testifies of our inability to assess their functional consequences on blood flow and on clinical outcome. The main indication of FFR is the assessment of stenoses with unclear hemodynamic significance. [45] As already discussed, pivotal animal studies by Gould et al. [3;4] have shown that flow starts to decline when a stenosis exceeds half of the reference diameter and therefore the 50% diameter stenosis has become the cornerstone of defining obstructive coronary artery disease. But data suggest that the 50% diameter stenosis might be inaccurate to be used for individual decision-making in real life patient population. [62;63]

As specified later, one of the goals of our work was to evaluate on a large population, whether any angiographic measure could be sufficiently accurate for the definition of ischemic potential of an individual lesion. When assessing functional severity, FFR is more accurate than exercise ECG, myocardial perfusion scintigraphy and stress echocardiography taken separately. Furthermore, in patients with ‘intermediate’ stenoses, the results of non-invasive tests are often contradictory, which renders appropriate clinical decision-making difficult. [58;64] As shown in the DEFER and the FAME 2 studies, the major adverse cardiac event rate of patients with angiographically intermediate stenoses, in whom revascularization was deferred due an FFR>0.80 is around 3% per year and this risk cannot be further decreased by PCI. [65-68]

- *Left main stem disease*

The left main is unique in many respects: (1) it is the largest coronary segment, perfuse the largest myocardial territory and its disease has major prognostic implications; (2) the left main is difficult to assess on angiography because it is ostial, short, and bifurcated; and (3) non-invasive assessment of the left main is clouded by many theoretical and practical limitations. FFR measurements in left main stenoses are feasible and reliable provided some precautions are taken.

Studies have shown that FFR is reliable for decision-making about revascularization in patients with left main stenosis, meaning that a lesion with FFR > 0.80 can be safely treated medically alone, while a lesion with FFR ≤ 0.80 requires revascularization: either surgically or interventional. [69]

Left main stenoses are often associated with additional stenoses in the left anterior descending and/or the left circumflex coronary arteries. The presence of a stenosis downstream limits hyperemic flow across the left main stenosis. The extent to which a second lesion affects the FFR measurement across a left main stenosis depends on the severity of the second lesion and of the myocardial mass supplied by that vessel. Yet in practice, it appears that the underestimation of the LM severity by the presence of a second stenosis in the LAD becomes clinically significant only in case of very tight LAD stenosis. [70]

- *Multivessel disease*

Patients with 'multivessel disease' represent a very heterogeneous population characterized by differences in clinical history, in comorbidities, in number of lesions, in their location and in their degree of complexity. The benefit of FFR-guided multivessel PCI compared with standard angiography guidance has been demonstrated in the FAME multicentric randomized clinical trial (Fractional Flow Reserve Versus Angiography for Multivessel Evaluation study). Patients in whom PCI was guided by FFR had significantly less 'hard events' (death and myocardial infarction) than patients in whom PCI had been guided solely on the basis of the angiogram. [71-72] These results were achieved at significantly lower cost and without prolonging the procedure. [73]

- *FFR and myocardial infarction*

Regarding the use of FFR in patients during or after myocardial infarctions one should distinguish several clinical settings.

In the setting of an ST-elevation acute myocardial infarction, there is no need for FFR measurements in the culprit artery. The clinical question is not whether the lesion is hemodynamically significant, and in addition, measurements performed in these circumstances would be of no value. During the acute phase of an acute coronary syndrome, myocardial dysfunction and oedema, microvascular stunning and spasm, thrombus embolization, etc. preclude microvascular dilation. These changes are dynamic in nature. Measurements related to the culprit vessel, obtained during the acute phase may be different from those obtained later, making FFR assessment of the culprit lesion potentially misleading. Several days and weeks after a myocardial infarction

occurs, the previously viable myocardium is partially replaced by scar tissue with a subsequent decrease of the myocardial mass to be perfused. [59] Accordingly, for an anatomically unchanged lesion, hyperemic flow and gradient will both decrease and FFR will increase. This principle is illustrated in **Figure 6**.

FFR measurements in the non-infarct artery reliably detect the ischemic potential even during primary PCI. It has been shown that FFR measurements in the non-culprit lesions during the acute phase of a myocardial infarction are similar to those obtained several weeks later. [74] This suggests that the diagnostic work-up of most patients admitted with an acute coronary syndrome could be entirely performed in the catheterization laboratory in one stage.

In the setting of non-ST elevation myocardial infarction the use of FFR measurement for the evaluation of the culprit lesion(s) is still debatable. However, in the

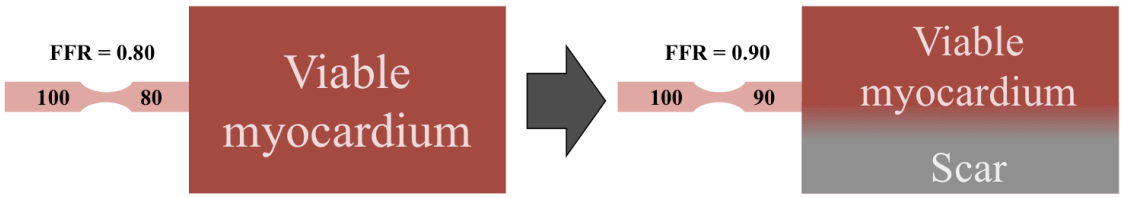

Figure 6 – Impact of old myocardial infarction on the functional relevance of a given stenosis. In case a coronary artery with a stenosis supplies a myocardial territory, containing scar due to former myocardial infarction, the blood flow is much smaller then normally. Accordingly, the transstenotic gradient will be smaller and FFR will be higher than if the supplied territory was completely healthy myocardial tissue, free from scar tissue.

FAMOUS-NSTEMI trial patients with non-ST elevation myocardial infarction were 1:1 randomized between angiography-guided versus FFR-guided revascularization. Although fewer patients underwent revascularization in the FFR-guided group, there was no difference in clinical outcome, including death, myocardial infarction, unplanned hospitalization or stroke during 12 months follow-up. [75]

- *Diffuse disease*

Coronary atherosclerosis is diffuse in nature and true isolated stenoses in an otherwise normal artery are exceptional. The concept of a focal lesion is mainly an angiographic

perception that does not reflect the underlying pathologic setting. Until recently, it was believed that when no focal narrowing of >50% was seen at the angiogram, flow and resistance were normal. It was therefore assumed that distal pressure was normal and thus that 'diffuse mild disease without focal stenosis' could not cause myocardial ischemia. This paradigm has recently been shifted thanks to better understanding by measuring FFR along the coronary artery: the presence of diffuse disease is often associated with a progressive decrease in coronary perfusion pressure and flow [52], which might even reach pathologic low values in the distality. This effect is difficult to predict from the angiogram. In contrast this decline in pressure correlates with the total atherosclerotic burden. [76] In approximately 10% of patients this abnormal epicardial resistance may be responsible for reversible myocardial ischemia. In these patients chest pain is often considered non-coronary because no single focal stenosis is found, and the myocardial perfusion imaging is wrongly considered false positive (actually a false ''false positive''). [77] Such diffuse disease and its hemodynamic impact should always be kept in mind when performing functional measurements Note, this diffuse disease is usually not correctable by PCI but it is responsible for the persisting low FFR values which are often found after otherwise successful stenting. In addition diffuse atherosclerosis goes along with abnormal vasomotion. [78]

- *Sequential stenoses*

When several stenoses are present in the same coronary artery, each of them will influence hyperemic flow and therefore the pressure gradient across the other one. The FFR can theoretically be calculated for each stenosis individually. [79;80] However, this is neither practical nor easy to perform. In practice, it is essential to keep in mind the 'hemodynamic cross talk' between the different stenoses. The influence of a distal stenosis on the more proximal is more pronounced than the reverse. A pull-back pressure recording under maximal hyperemia is the best way for identifying the exact location and physiological significance of sequential stenoses individually.

- *FFR in bifurcation lesions*

Bifurcation stenoses are particularly difficult to be evaluated on angiography. The principle of FFR-guided PCI applies logically also in bifurcation lesions even though separate clinical outcome data are currently still limited. The use of FFR for decision-

making in terms of complexity of bifurcation PCI has been thoroughly investigated by Koo et al. [81-83] The results of these studies can be summarized as follows: (1) After stenting the main branch, the jailed ostium of the side branch is often narrowed at angiography. Such stenoses are grossly overestimated by angiography: Koo et al. found that none of the ostial lesions with a diameter stenosis <75% were found to have an FFR below 0.75. (2) When performing kissing balloon dilation only in ostial stenoses with an FFR <0.75, the FFR at 6 months was >0.75 in 95% of all cases.

- *Small vessels*

Angiographic evaluation of lesion severity in small vessels is difficult, not only because of the resolution derived intrinsic inaccuracy of angiographic measures, but also due to the small supplied myocardial mass. On the other hand, considering the poor performance of current revascularization techniques in small vessels, unnecessary intervention should be avoided. As shown by Puymirat et al., compared to angiographic guidance FFR-guided revascularization of small vessels is associated with improved long-term clinical outcome, mainly driven by significant benefit in non-fatal myocardial infarction and target vessel revascularization. [84]

- *Post-Intervention FFR*

After successful stenting no noticeable hyperemic gradient should be present across a well-deployed stent. [85] Even though malapposed struts might induce turbulent flow, FFR is not a good tool for identifying malapposition or underexpansion of the stent. However, the hyperemic pressure pull-back recording is an informative tool for assessing the extent and significance of residual ischemia, induced within-, proximal- or distal to the stent. [86;87]

- *FFR for indicating bypass grafting*

Data about FFR-guided surgical revascularization are limited. An observational study showed that graft occlusion rate is twice as high at one year when the graft was placed on a vessel with functionally non-significant lesion, than when placed on a vessel with functionally significant stenosis. There was also a trend suggesting that this hold true for arterial grafts. [88] Until now the clinical consequences of FFR-guidance prior bypass surgery was examined only in a retrospective registry. Our working group showed that patients with angiographically similar coronary artery disease severity are

receiving markedly less grafts, when at least one intermediate lesion is treated according to the value of FFR (deferred or grafted), as compared to the purely angio-guided controls. Of note, the lower number of grafts was associated neither with potential hazard in terms of death, myocardial infarction or target vessel revascularization nor with more symptoms during 5 years follow-up. [89]

Better understanding can be expected after the results of GRAFFITI trial (NCT01810224), where patients are randomly assigned to FFR-guided versus angiography-guided surgical revascularization.

Similarly, the FAME 3 trial (NCT02100722) investigates the potential benefits of FFR guidance in angiographically severe multivessel disease, assigning patients randomly to FFR-guided percutaneous revascularization versus angiography-guided surgical revascularization.

- *FFR for revascularization in sclerotic bypass grafts*

Data from a retrospective registry suggest the beneficial effect of FFR-guided revascularization of sclerotic bypass grafts. An FFR-guided PCI strategy of coronary bypass grafts was found to be associated with significantly lower rate of major adverse cardiac and cerebrovascular event at long-term follow-up, as compared to angiographic guidance. Additionally, procedural costs were found to be significantly lower, as well. [90]

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4. Objectives

As described above in details, FFR has opened a novel approach to functional assessment of coronary artery stenoses, and it has become the standard of reference to define the ischemic potential of epicardial stenoses of intermediate angiographic severity based on outcomes of randomized trials.

Despite powerful outcome data and the highest level of recommendation by European revascularization guidelines the adaptation by the interventional cardiologists community is still limited. As the results of *International Survey on Interventional Strategy* have showed, interventional cardiologists are still prone to make decisions about intermediate stenoses purely on the basis of its angiographic appearance in almost three quarter of all cases, even when non-invasive proof of ischemia is missing, and even, when the use of additional invasive diagnostic tools is not restricted by financial limitations. **Figure 7.** Although the ESC class I recommendation for FFR would apply to all 12 lesions in our survey, given their intermediate nature and absence of prior functional testing, no respondent selected FFR for 100% of the cases. A single respondent out of 495 (0.2%) selected FFR in 10 of the 12 cases (the highest FFR user among all), while 133 of the 495 Participants (27%) never selected FFR at all, indicating a major disconnect between recommendations and true clinical practice. Decisions were still dominantly based on pure angiographic appearance with questionable validity. [61]

What can be the cause in the background? Since the survey was designed to eliminate all the potential extrinsic burdens, we have to believe that the disconnect between practice and guidelines is due to remaining uncertainties, if any. Indeed, there might be some important questions regarding the concept, causing potential hesitation against its unlimited clinical applicability. Which can be these questions, inducing uncertainties? In the initial validation studies FFR was measured during left and right heart catheterization, as right atrial pressure was added in the computation of FFR_{myo}, as explained in details above. Currently, as being the routine tool of interventional practice, FFR is measured during standard coronary angiography by positioning a pressure wire across the coronary stenosis, and calculated as the simple ratio of distal coronary pressure (P_d) and aortic pressure (P_a) during stable and maximal hyperemia without accounting for the right atrial pressure (P_{ra}). This simplification assumes that right atrial

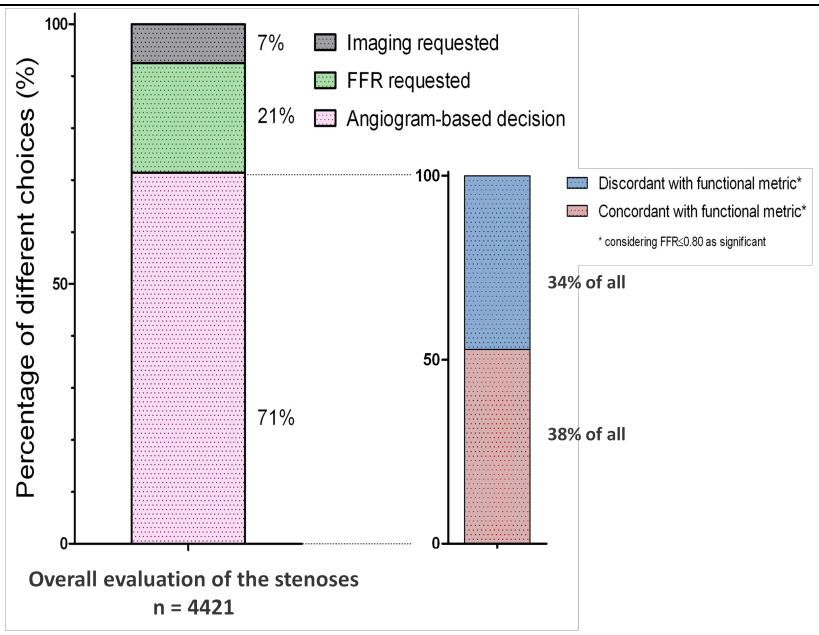

Figure 7 - Distribution of different decisions and the appropriateness of purely angiogram-based decisions in International Survey on Interventional Strategy. Left panel shows the distribution of decisions over the entire dataset: In 7% of all evaluations an imaging modality (quantitative coronary angiography, intravascular ultrasound or optical coherence tomography) was requested and in 21% the need for FFR was expressed. In the rest 71% angiography was found sufficient by the Participants to decide about significance. Among the latter, the proportion of concordance and discordance with the known functional metric is depicted in the right panel. *Adapted from Toth G et al. [61]*

pressure can be neglected as it has a magnitude difference as compared to the arterial values and so it would have limited impact on the calculated FFR value. Ever since, this assumption has been confirmed and supported by the excellent clinical outcome of patients managed according to the simplified FFR measurement. All the clinical outcome data obtained after FFR-guided decision-making are based on the simple P_d/P_a ratio, during maximal hyperemia. Deferring intermediate coronary stenoses of stable patients with single vessel disease and FFR above 0.75 has been demonstrated to be safe and associated with excellent clinical outcome up to 15 years [65]. In the FAME and FAME 2 studies a safer cut-off value of 0.80 has been adopted to take into account the intrinsic variability of FFR. Here, an FFR value higher than 0.80 has shown a

negative predictive value over 95%, and FFR-guided revascularization has resulted into a superior clinical outcome as compared with medical therapy or with angiography guided revascularization strategies [66;67;71;72]. These evidences, along with its ease of measurement, have facilitated a growing adoption and broadening field of indication for FFR assessment of coronary stenoses.

As field of application got wider, debates have risen again about FFRs' universal applicability and validity including patients with elevated right atrial pressures, such as patients with myocardial or valvular heart failure. [48;49] Accordingly, this question requires clarification.

Similarly, as the cornerstone of FFR measurement is the reliable and stable maximal hyperemia, its proper induction is also recurrently debated. While studies of intracoronary adenosine doses and Doppler flow velocity evaluation exist in the literature, no prior study has created sufficiently detailed and convincingly extensive dose-response curve in patients. Therefore the sense or non-sense of applying further increased, extreme dosages of adenosine is a recurring issue, requiring clarification. The original work applying adenosine to the human coronary circulation recorded Doppler velocity response in 33 arteries for a lower range of intracoronary adenosine from 2 to 16 μg only, using intracoronary papaverine as the comparator. [91] They observed that 16 μg produced hyperemia within 10% of papaverine in 90% of patients, consistent with our findings of a large increase in flow at even low doses of intracoronary adenosine but submaximal in some cases. A smaller study monitored Doppler velocity in 12 patients starting at 50 μg of intracoronary adenosine then increasing until a maximal response or side effects. [92] Their mean dose of intracoronary adenosine that produced maximum hyperemia was 230 μg. A larger study of 457 patients found a significant increase in Doppler flow velocity with increasing doses of adenosine, albeit between two modest doses of intracoronary adenosine (average 24 μg versus 35-36 μg). [93] **Table 2** summarizes 18 published studies over 15 years measuring FFR in 1,294 lesions using either serial doses of intracoronary adenosine or comparing intracoronary to intravenous adenosine. [50;94-110] With rare exceptions, all studies found no significant decrease in FFR with intracoronary adenosine doses beyond 100 to 210 μg and/or demonstrated equivalence between intracoronary and intravenous administration.

Table 2 – Definition of optimal dosage of adenosine. Studies in the literature, investigating optimal dosing of intracoronary adenosine for the measurement of FFR

1st author	Lesions	IC doses (μg)	Summary
De Bruyne et al. [94]	21	20 and 40	No difference vs intravenous adenosine
Jeremias et al. [95]	60	varied 15-24	Δ=+0.004±0.03 vs intravenous adenosine
Murtagh et al. [96]	215	serial 12 to 48	Minimum FFR started at 42 μg
Casella et al. [97]	36	serial 16 to 40	No significant difference among doses
Rzeczuch et al. [98]	53	30, 60, and 90	No further decrease after 60 μg
López -Palop et al. [99]	60	serial 15 to 210	17% of lesions needed 210 μg for minimum
Casella et al. [100]	50	serial 60 to 150	150 μg no different than intravenous adenosine
Koo et al. [101]	50	40/80 (right/left)	Inferior to intravenous adenosine
Aarnoudse et al. [102]	30	40	No different than intravenous adenosine
Rioufol et al. [103]	108	40, 100, and 150	No further decrease after 100 μg
Yoon et al. [104]	44	varied 36-80	Inferior to intravenous adenosine Δ=+0.023
De Luca et al. [50]	50	serial 60 to 720	No further decrease after 180 μg
Leone et al. [105]	45	60, 300, and 600	600 μg no different than intravenous adenosine
López-Palop et al. [106]	108	serial 60 to 600	180 μg no different than intravenous adenosine
Sandhu et al. [107]	56	60, 100, and 120	No different than intravenous adenosine
Khashaba et al. [108]	30	150	No different than intravenous adenosine
Wang et al. [109]	40	40 and 60	No difference between doses
Lim et al. [110]	238	40/80 (right/left)	93% agreement with intravenous adenosine

As shown debates are still rising against some building stones of FFR, such as (1) its disposability by angiographic metrics, (2) its applicability in patients with heart failure and elevated filling pressure values or (3) the proper way of measurement concerning the induction of hyperemia. Therefore the aims of this work and their perspectives are described as follows:

(1) In daily practice the vast majority of decisions about revascularization are based on diameter stenosis (DS) as gauged by visual estimation on coronary angiogram. Anatomic severity on quantitative coronary angiography (QCA) is limited or 'one dimensional' oversimplified measure of stenosis severity that do not account for all aspects of severity, especially for 'intermediate' stenosis. **Accordingly, the first goal of the present work is to analyze the concordance or discordance between stenosis severity by QCA and by FFR in a large unselected patient cohort. (This topic will be referred as *QCA vs FFR study*)**

(2) As described above, since in the majority of patients with coronary artery disease right atrial pressure is low, the latter is neglected in the calculation of FFR and the ratio of mean distal coronary pressure and mean aortic pressure at maximum hyperemia is simply called FFR. The omission of right atrial pressure simplifies FFR calculations during routine diagnostic coronary angiography.

Yet, whether FFR measurement is still reliable across a wide range of hemodynamic conditions, e.g. in patients with heart failure, is not clear and therefore a frequent target for criticism. Accordingly, the second goal of this work was to assess the impact, if any, of a wide range of right atrial pressures on FFR assessment and on FFR-guided clinical decision-making. (This topic will be referred as *FFR vs FFR_{myo} study*)

(3) As described in details above, FFR relates the current maximum blood flow in a stenotic artery to the potential maximum blood flow in absence of the lesion. Note, only under conditions of *maximal* hyperemia does the pressure ratio between the distal coronary artery and aorta equal the maximum flow ratio between stenotic and normal conditions, therefore reliable induction of *maximal* hyperemia is crucial for accurate measurement. For a variety of reasons, intracoronary adenosine has been used more commonly in daily practice and in the clinical literature. Despite this widespread adoption of intracoronary adenosine, a recurring debate still exists regarding its optimal dose. **Therefore, the third goal of this work is to define the dose-response relationship between intracoronary adenosine and its resulting hyperemia. (This topic will be referred as *Dose-response study*)**

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The three main studies of this work are logically well linked by (1) assessing the discrepancies between morphological appearance of a given coronary stenosis and true functional relevance, accordingly emphasizing the indication for consequent use of FFR in clinical practice (*QCA vs FFR study*); (2) evaluating the conceptual validity of the applied formula (*FFR vs* FFR_{myo} *study*); and (3) defining the proper practice for accurate measurement (*Dose-response study*). But as they are markedly different in terms of population size, methodology, statistical methods etc., for didactic reasons they will be presented separately over the *Methods* and *Results* paragraphs.

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5. Methods

5/1 – Methods for QCA vs FFR study

5/1.1 Study population

Between September 1999 and December 2011, 37.047 coronary angiograms and 14.989 PCI's were performed in the Cardiovascular Center Aalst, Aalst, Belgium. Among them, 2.986 patients (appr. 1:5 relation as compared to PCI's) underwent both QCA and FFR measurements in at least one stenosis. Only stable coronary stenoses were considered (patients with stable CAD, or the non-culprit vessels of patient with acute CAD). Repeated measurements of the same lesion in the same setting were excluded. Measurements of multiple stenoses in the same patient could be included. These data were stored prospectively in the local database together with the clinical characteristics and constitute the basis of the present analysis. All subjects gave written informed consent approved by the local ethics committee prior to undergoing coronary angiography.

5/1.2 Coronary angiography

Coronary angiography was performed by a standard percutaneous femoral or radial approach with a 6 or 7Fr diagnostic- or guiding catheter. After administration of 200-300 μg intracoronary isosorbide dinitrate, the angiogram was repeated in the projection allowing the best possible visualization of the stenosis.

QCA was performed based on the technology, described previously, [111;112] using one of the following software: Siemens Healthcare Axiom Artis VB35D110803 (Siemens Medical Solutions, Siemens AG; Forcheim, Germany); Siemens Healthcare ACOM.PC 5.01 System (Siemens Medical Solutions, Siemens AG; Forcheim, Germany); General Electric AW VolumeShare 6E (General Electric Inc., Fairfield, Ohio, US). All measurements were obtained by an experienced technician, unaware of the FFR results. Data were introduced on a different page of the database. The projection was chosen to avoid, as far as possible, foreshortening or overlap of other arterial segments. The contrast-filled catheter was used for calibration. From an end-diastolic still-frame, reference diameter (RD, mm), minimum luminal diameter (MLD,

mm), percent diameter stenosis (DS, %) and lesions length were calculated. The coronary arterial segments were defined according to the American Heart Association and modified for the ARTS I and II studies. [113] Segment 5 corresponds to the left main stem (LM), and segments 4, 10, 13, 14, 15, 16 and 17 were considered 'distal'.

5/1.3 Fractional flow reserve measurement

FFR was measured as above described in details. Briefly, after intracoronary administration of isosorbide dinitrate (200 μg), a pressure monitoring guide wire (St. Jude Medical Inc., St. Paul, Minnesota, US) was advanced distal to the coronary artery stenosis. Hyperemia was obtained after administration of intravenous (continuous infusion of 140 μg/kg/min, 18% of all cases) or intracoronary (bolus of 50-150 μg, 79% of all cases) adenosine or intracoronary Papaverine (bolus of 10-20 mg, 3% of all cases). FFR was defined as the ratio of the simultaneously recorded mean arterial pressure distal to the stenosis and the mean aortic pressure at the tip of the guiding catheter during stable, steady state hyperemia. An FFR value ≤0.80 was considered 'positive', i.e. likely to induce reversible myocardial ischemia. An FFR value >0.80 was considered 'negative', i.e. unlikely to induce reversible myocardial ischemia.

5/1.4 Statistical Analysis

All analyses were performed with Prism GraphPad 5.0 (GraphPad Software Inc., California, US) and SPSS 20.0 (IBM Inc., New York, US). Summary descriptive statistics are reported as mean ± standard deviation, median (inter quartile range) or counts (%), as appropriate. 95% confidence intervals (CI) are added, as appropriate. Normal distribution was tested with the D'Agostino-Pearson omnibus K2 test. Correlation among variables was determined by Pearson or Spearman correlation tests, as appropriate and expressed in r value. Sensitivity, specificity, diagnostic accuracy, and optimal diagnostic cut-off value were defined from the calculated receiver operator characteristic curves, as appropriate. Receiver operator characteristics curves were compared as described by Hanley et al. [114] Optimal diagnostic cut-off value was defined based on the Youden's index, calculated as [(sensitivity + specificity) – 1],

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namely where the sum of sensitivity and specificity is maximized. Logistic regression analysis was performed to assess the impact of various clinical and anatomical characteristics on the accuracy of 50% DS cut-off value in predicting FFR ≤ 0.80. P for interaction was calculated within all subgroups, as appropriate. A probability value of $p<0.05$ was considered statistically significant.

5/2 – Methods for FFR vs FFR_{myo} study

5/2.1 Study population

Between 1997 and 2013 approximately 45.000 coronary angiograms were performed in the Cardiovascular Center Aalst, Aalst, Belgium. Among them 1.235 patients (~3%) underwent both left- and right heart catheterization *and* FFR measurement in at least one coronary stenosis, as indicated by operators discretion. Data were stored prospectively in the local database together with the clinical characteristics and constitute the basis of the present analysis. All subjects gave written informed consent to the use of anonymized clinical data for research purposes.

5/2.2 Left and right heart catheterization

Procedures were performed by a standard percutaneous femoral approach, 6F diagnostic catheters were used for injecting the coronary arteries and obtaining pressure values in the left heart, including aortic and left ventricular pressures, 8F Swan-Ganz catheter was used for obtaining pressure values in the right heart, including right atrial-, right ventricular-, pulmonary arterial- and pulmonary capillary wedge pressures.

5/2.3 Fractional flow reserve measurement

We measured and calculated FFR and FFR_{myo} for every coronary stenosis in the range between 30-90% diameter stenosis by visual estimate. FFR was measured as above described in details. Briefly, a calibrated pressure monitoring guide wire (PressureWire^{TM}, St. Jude Medical Inc., St. Paul, Minnesota, US) was advanced distal to the coronary artery stenosis. After an intracoronary bolus of isosorbide dinitrate

(200 μg), maximal hyperemia was induced by either intravenous infusion (140 μg /kg/min) or an intracoronary bolus (>100 μg) of adenosine.

FFR was calculated as follows:

$$\mathrm{FFR} = \frac{\mathrm{Pd}}{\mathrm{Pa}}$$

where P_d is the mean arterial pressure distal to the stenosis, and P_a is the mean aortic pressure at the tip of the guiding catheter during stable, steady state hyperemia.

FFR_{myo} was calculated offline as follows:

$$\mathrm{FFRmyo} = \frac{\mathrm{Pd} - \mathrm{Pra}}{\mathrm{Pa} - \mathrm{Pra}}$$

where P_{ra} is the mean right atrial pressure.

5/2.4 Statistical Analysis

All analyses were performed with Prism GraphPad 5.0 (GraphPad Software Inc., California, US). Summary descriptive statistics are reported as mean ± standard deviation, median (inter quartile range) or counts (%), as appropriate. Normal distribution was tested with the D'Agostino-Pearson omnibus k2 test. Unpaired t-test or Mann-Whitney test were used to compare two independent groups, as appropriate. To compare multiple groups one-way ANOVA or Kruskal-Wallis test were used, as appropriate. Correlation between variables was determined by Pearson- or Spearman correlation tests, as appropriate. Sensitivity, specificity, diagnostic accuracy, and optimal diagnostic cut-off value were defined from the calculated receiver operator characteristic curves, as appropriate. A probability value of $p < 0.05$ was considered statistically significant.

5/3 – Methods for Dose-response study

5/3.1 Study population

Patients with stable CAD undergoing routine diagnostic coronary angiography for a variety of indications were approached for participation between April and November of 2014. All patients had documented coronary atherosclerosis, but the measurements

were performed in vessels free of any stenosis with more than 20% diameter reduction. Each subject provided written informed consent as approved by the institutional ethics committee.

5/3.2 Intracoronary Doppler velocity measurement

Following standard diagnostic coronary angiography, 200 μg of intracoronary nitroglycerin was administered to minimize epicardial vasomotor tone. Then a 0.014" Doppler wire (FloWire, Volcano Corporation, San Diego, California, USA) was introduced via a 6 Fr guiding catheter into the target coronary artery and positioned under fluoroscopy to obtain an optimal and stable flow velocity signal. In all patients, the guide wire was manipulated to place the Doppler sensor facing the oncoming coronary flow.

First, resting Doppler velocity was measured and recorded for at least 1 minute to ensure a steady-state baseline. Next, Doppler velocity was measured and recorded for at least 1 minute after an 8mL intracoronary bolus administration of arterial blood, saline at room temperature, contrast medium (iodixanol 270 mg/mL), 9 escalating doses of adenosine (4, 12, 20, 60, 100, 160, 200, 300, and 500 μg), and finally a mixture of 200 μg of adenosine plus contrast medium. For the sake of this protocol, the adenosine solution prepared by the pharmacy contained 100 μg/mL and the dilutions were adjusted to reach 8 mL for all injections. In order to obtain optimal flow velocity tracings, we elected not to flush the 0, 60, 100, 160, 200, 300, and 500 e after an 8mL r guiding catheter into the target coronary artery and ximately 1.5 to 2 seconds). At the end of the measurements performed after administration of contrast material the remaining contrast was removed from the catheter prior to the next injection.

After each intracoronary administration, no further injection was performed for 2 minutes to allow the Doppler velocity to return to its baseline value. Hemodynamic parameters of heart rate and mean aortic pressure were recorded for each Doppler velocity measurement. AV-block was defined as at least one P wave, not followed by QRS. In case of AV-block, heart rate was defined in the post-block phase. **Figure 8** depicts a typical Doppler velocity tracing and indicates the indices measured for each intracoronary bolus. We defined the plateau hyperemic period as the time during which

flow velocity reached at least 95% of its maximum. The time needed to come back to baseline was defined by the return to less than 10% above the starting value.

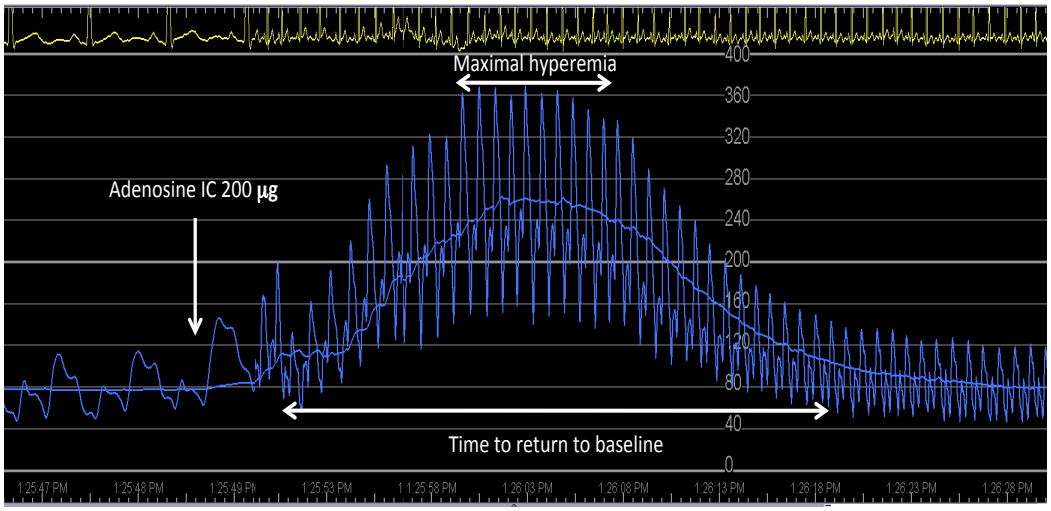

Figure 8 – Example of a typical Doppler velocity tracing. The figure illustrates, how the various measurements have been performed in the present study. First, resting Doppler velocity was measured and recorded for at least 1 minute to ensure a steady-state baseline. Next, the predefined medium was injected heart rhythm and hemodynamic parameters of heart rate and mean aortic pressure were recorded for each Doppler velocity measurement. In order to obtain optimal flow velocity tracings, we elected not to flush the 'dead space'. This allowed to minimizing the duration of interruption of the aortic pressure signal (approximately 1.5 to 2 seconds). After each intracoronary administration, no further injection was performed for 2 minutes to allow the Doppler velocity to return to its baseline value.

5/3.3 Model for FFR dependence on adenosine dose

The following simulation was applied to investigate, whether the potential deviations of measured maximal flow (Q_{max}) from true maximal flow can have any clinically relevant impact on the measured FFR value. Mathematical background of the simulation is described below.

To translate the intracoronary adenosine dose into its effect on FFR, a model based on standard coronary physiology linked the degree of hyperemia to the relative distal

coronary pressure (P_d/P_a). It started with the classic equation for pressure loss versus flow relationship for a vascular stenosis

$$\Delta P = P_a - P_d = C_v * Q + C_e * Q^2$$

then transformed it into a more portable, unitless form

$$P_d/P_a = 1 - [C_v*Q_r/P_a] * (Q/Q_r) - [C_e*Q_r^2/P_a] * (Q/Q_r)^2$$

where C_v and C_e are the viscous and expansion coefficients that depend on vessel and stenosis geometry, P_d is the distal coronary pressure, P_a is the proximal coronary pressure, Q_r is the resting flow, and Q is the current flow. At resting condition $Q/Q_r=1$ and P_d/P_a is can be called as '*resting* P_d/P_a'. At maximum hyperemia Q/Q_r is *per definitionem* the coronary flow reserve (CFR) and P_d/P_a is *per definitionem* the FFR.

For simulation, specific values were chosen for resting $P_d/P_a = 0.93$ and for FFR = 0.79 based on the median values from 1,593 lesions assessed by pressure wire [115] and CFR = 2.0 based on the weighted average from 1,118 lesions assessed by Doppler wire in the literature. [116] Using these values in the model yielded a relationship between P_d/P_a and percentage of maximum hyperemia starting at 0% (rest, $Q/Q_r=1$) and ending at 100% (hyperemia, Q/Q_r=CFR).

5/3.4 Statistical analysis

Analyses were performed using Prism GraphPad 5.0 (GraphPad Software, California) and R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) with standard summary statistics. Applicable tests were two-tailed and $p<0.05$ was considered statistically significant.

An ANOVA model with mixed effects (to account for repeated measurements from the same subject) tested for a significant interaction between contrast and adenosine in their 2-by-2 factorial design: baseline, contrast, 200 μg adenosine, and both together. Similarly, an ANOVA mixed-effects model compared Doppler velocity among the 3 viscosity conditions (saline, contrast, and blood). If an overall ANOVA p-value was significant, then a Tukey all-pair comparison was applied to determine which conditions provided a different response.

Dose-response analysis was performed in two ways. First, an ANOVA mixed-effects model with potential Tukey all-pair comparison analyzed the flow response over 10

conditions (baseline plus 4, 12, 20, 60, 100, 160, 200, 300, and 500 μg intracoronary adenosine). Flow response was assessed by the normalized flow, a unitless ratio Q/Q_{max}, where Q equals the Doppler velocity and Q_{max} represents the largest observed Doppler velocity in response to intracoronary adenosine. Hemodynamic response assessed both heart rate and mean arterial pressure.

Second, a model-based approach used an explicit formula for the relationship between intracoronary adenosine dose and normalized flow (Q/Q_{max}). Because adenosine follows an enzymatic reaction to produce hyperemia, it makes physiologic sense to employ the Michaelis-Menten model for enzyme kinetics. Two minor modifications were necessary to customize the general model for the specifics of intracoronary adenosine hyperemia. Because Q/Q_{max} approaches a maximum value of 1 at high/infinite adenosine concentration and equals >0 at baseline due to endogenous adenosine in the coronary circulation, our customized model was

$$Q/Q_{max} = (dose+offset) / (k + [dose+offset]),$$

where the constant "k" describes when Q/Q_{max} equals 50% and the constant "offset" adjusts for baseline, physiologic adenosine. The variable "dose" equals the intracoronary adenosine amount in μg. The R package lme4 was used for non-linear fitting of the model to the data. Because a mixed effects model (to account for repeated measurements from the same subject) produced similar results to a fixed effects model (not accounting for repeated measurements from the same subject), results and figures employ the fixed effects model given more robust and accepted techniques for its confidence intervals.

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6. Results

6/1 – Results for QCA vs FFR study

Data from 4.086 coronary artery stenoses in 2.986 patients were analyzed. Patients' clinical characteristics are summarized in **Table 3**. FFR value was in median 0.82 (0.74; 0.88) DS was 48% (39; 57) and MLD was 1.40 mm (1.10; 1.71).

Table 3 – Detailed clinical characteristics of the investigated patient population

	(n = 2986)
Age; mean in years ± SD	66.4 ± 10.4
Male gender; n (%)	1891 (63)
Hypertension; n (%)	1681 (56)
Hypercholesterolemia; n (%)	1852 (62)
Diabetes mellitus; n (%)	1078 (36)
Body-mass-index; mean ± SD	26.3 ± 10.3
Smoking; n (%)	1127 (38)

6/1.1 Overall relationship between angiographic metrics and FFR

The relationship between DS and FFR was only modest but statistically significant (-0.38 [95% CI: -0.41; -0.36]; p<0.001) with marked scatter around the regression line. **Figure 9 – Panel A**. A DS ≥50% correctly identified an FFR value ≤ 0.80 with a sensitivity of 61% [95% CI: 59; 63] and a specificity of 67% [95% CI: 65; 69], associated with a diagnostic accuracy of 0.64 [95% CI: 0.56; 0.72].

Similarly, the relationship between MLD and FFR was statistically significant (0.45 [95% CI: 0.42; 0.47] p<0.001) with a large scatter of the data.

6/1.2 Influence of patients' characteristics

Table 4 shows the stratified analysis of the sensitivity, specificity, diagnostic accuracy and positive and negative likelihood ratios belonging to 50% DS for predicting an FFR

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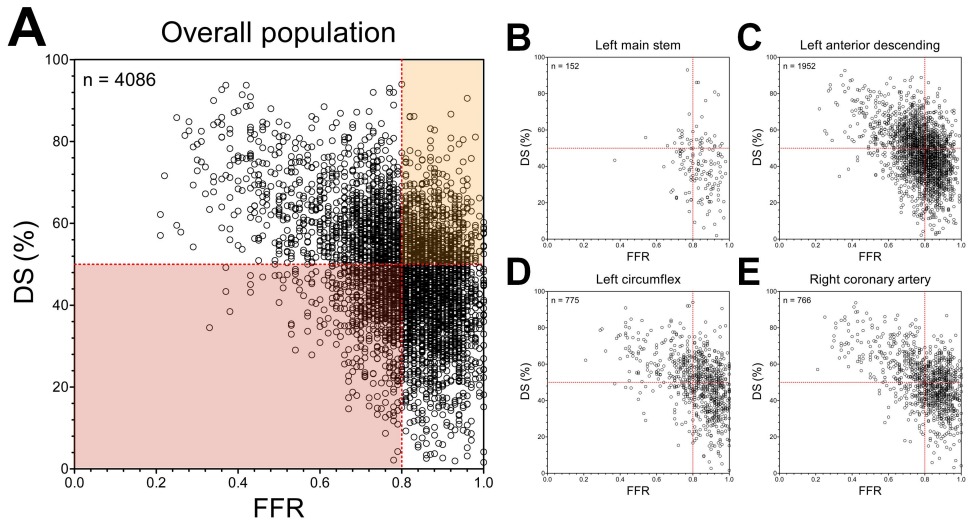

Figure 9 – Correlation between Diameter Stenosis versus Fractional Flow Reserve (FFR). Correlation is assessed according to different lesion locations, namely in the overall population (Panel A) and specifically in the left main stem (Panel B) and the three major branches (Panel C-E). The x-axes indicate the functional metric (FFR), the y-axes indicate the angiographic metric (DS).

value ≤ 0.80 according to various patient characteristics. Two parameters, namely male gender (p=0.017) and presence of diabetes (p=0.005) negatively influenced the value of 50% DS in predicting significant FFR.

6/1.3 Influence of lesion characteristics

Table 5A shows the stratified analysis of the sensitivity, specificity, diagnostic accuracy and positive and negative likelihood ratios of 50% DS cut-off value for predicting an FFR value ≤ 0.80 according to lesion characteristics.

6/1.4 Diagnostic performance of 50% versus 70% DS

Table 5B shows the stratified analysis of the sensitivity, specificity, diagnostic accuracy and likelihood ratio of 70% DS cut-off value for predicting an FFR value ≤0.80 according to lesion characteristics. **Figure 10** shows the Youden's index for the 50%

Table 4 – Diagnostic accuracy of diameter stenosis and clinical characteristics. Stratified analysis of the diagnostic accuracy of diameter stenosis for predicting an FFR value ≤ 0.80 according to different patients' characteristics. Cut-off value for diameter stenosis was considered to be 50%. * p for interaction;

Categories		Sensitivity	Specificity	Accuracy		p*
	n(%)	% [95% CI]	% [95% CI]	AUC [95% CI]	OR [95% CI]	
Age						
≥65ys	2241 (55)	63.8 [60.1; 66.9]	66.0 [63.3; 68.6]	0.65 [0.63; 0.67]	3.557 [2.984; 4.240]	
<65ys	1845 (45)	58.6 [55.3; 61.9]	68.3 [65.3; 71.3]	0.64 [0.61; 0.66]	3.021 [2.497; 3.655]	0.215
Gender						
Male	2611 (64)	60.1 [57.3; 62.7]	65.9 [63.3; 68.5]	0.63 [0.61; 0.65]	2.916 [2.486; 3.419]	
Female	1475 (36)	64.2 [60.2; 68.1]	68.6 [65.4; 71.6]	0.66 [0.64; 0.69]	4.071 [3.260; 5.083]	**0.017**
Hypertension						
Yes	2320 (57)	61.0 [58.0; 63.9]	65.1 [62.4; 67.8]	0.63 [0.61; 0.65]	2.978 [2.514; 3.528]	
No	1766 (43)	61.8 [58.4; 65.2]	69.4 [66.4; 72.3]	0.66 [0.63; 0.68]	3.696 [3.032; 4.505]	0.105
Hypercholesterolemia						
Yes	2575 (63)	60.2 [57.4; 63.0]	68.1 [65.6; 70.6]	0.64 [0.62; 0.66]	3.248 [2.762; 3.820]	
No	1511 (37)	63.3 [59.6; 66.9]	65.2 [61.8; 68.4]	0.64 [0.61; 0.67]	3.327 [2.691; 4.113]	0.857
Diabetes mellitus						
Yes	1488 (36)	57.2 [53.6; 60.8]	65.8 [62.2; 69.2]	0.62 [0.59; 0.64]	2.589 [2.099; 3.194]	
No	2598 (74)	64.1 [61.2; 66.9]	67.6 [65.2; 70.0]	0.66 [0.64; 0.88]	3.808 [3.231; 4.487]	**0.005**
Smoking						
Yes	1710 (42)	59.7 [56.2; 63.1]	67.7 [64.5; 70.7]	0.64 [0.61; 0.66]	3.104 [2.546; 3.785]	
No	2376 (58)	62.6 [59.6; 65.5]	66.5 [63.9; 69.1]	0.65 [0.62; 0.67]	3.396 [2.868; 4.022]	0.500
Family history						
Yes	549 (13)	63.4 [57.2; 69.2]	70.0 [64.4; 75.3]	0.67 [0.62; 0.71]	4.096 [2.867; 5.851]	
No	3537 (87)	61.0 [58.6; 63.4]	66.6 [64.4; 68.7]	0.64 [0.54; 0.73]	3.158 [2.751; 3.625]	0.184
Body mass index						
25kg/m^2≤	2695 (66)	62.0 [59.3; 64.7]	65.9 [63.3; 68.3]	0.64 [0.62; 0.66]	3.241 [2.768; 3.795]	
25kg/m^2>	1210 (30)	61.3 [57.0; 65.5]	69.6 [66.0; 73.1]	0.66 [0.62; 0.69]	3.642 [2.867; 4.628]	0.424

DS cut-off versus 70% DS cut-off for the various subsets of lesions. The overall diagnostic performance of angiography is significantly weaker when a 70% DS is considered as cut-off value (0.30 (0.28; 0.32) vs 0.08 (0.06; 0.12), respectively; p=0.004). Specifically, Youden's index decreased from 0.28 to 0.11 for the overall population, and showed an absolute decrease of 0.16 ± 0.05 in the various anatomical subsets.

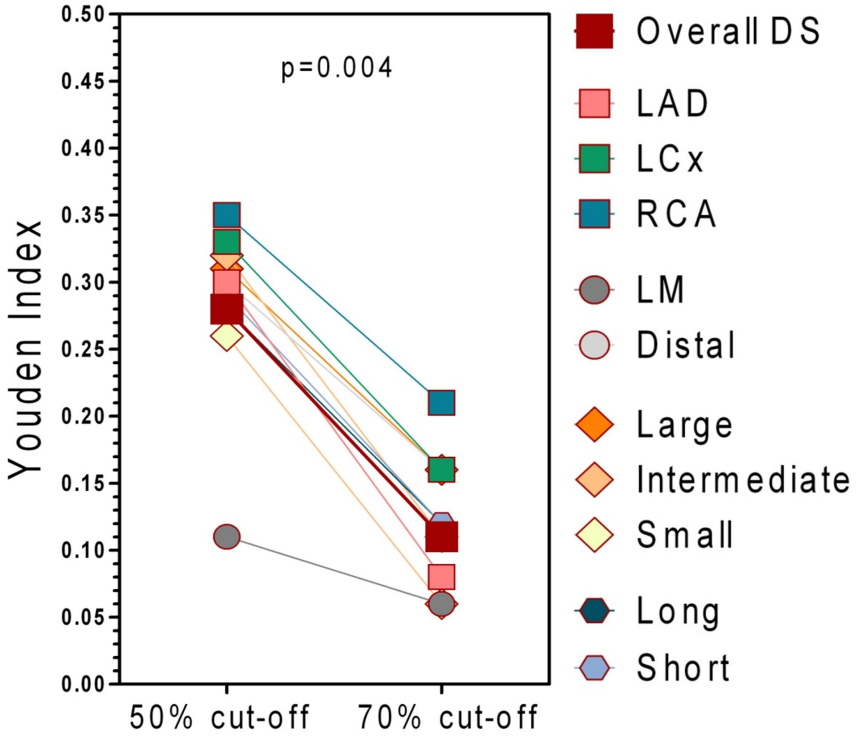

Figure 10 – Diagnostic performance of different angiographic cut-off values. Youden's index for the 50% DS cut-off versus 70% DS cut-off in various anatomical subsets of lesions.

6/1.5 Optimal angiographic cut-off values for percent diameter stenosis

The diagnostic accuracy and the corresponding optimal cut-off values were defined for several, clinically relevant anatomical settings. Detailed analysis is shown in **Table 6**. Comparison between LM (n=152), the overall population (n=4086) and the distal segments (n=472) is depicted in **Figure 11**. The optimal cut-off values of DS for predicting FFR $\leq$ 0.80 were markedly different: 43% for the LM, 51% for the global population, 55% for the small vessels. However, the corresponding accuracies remained low for all the three groups (0.65 [95% CI: 0.56; 0.74] vs 0.69 [95% CI: 0.60; 0.78] vs 0.72 [95% CI: 0.67; 0.77], respectively). The corresponding optimal cut-off values of MLD are 1.6 mm, 1.5 mm and 1.1 mm, respectively.

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Table 5 – Diagnostic accuracy of diameter stenosis and lesion characteristics.

Stratified analysis of the diagnostic accuracy of diameter stenosis for predicting an FFR value ≤ 0.80 according to different lesions characteristics. Cut-off value for diameter stenosis was considered to be 50% in **Panel A** and 70% in **Panel B**.

A - Diameter stenosis cut-off value is set to 50%					
Categories n (%)	**Sensitivity** % [95% CI]	**Specificity** % [95% CI]	**Accuracy** AUC [95% CI]	**LR+**	**LR-**
Localization					
Overall					
4086 (100)	61.2 [59.0; 63.4]	66.9 [64.9; 68.8]	0.64 [0.56; 0.72]	1.87	0.57
LAD					
1952 (48)	55.5 [52.5; 58.4]	74.2 [71.1; 77.1]	0.65 [0.62; 0.67]	2.12	0.60
LCx					
775 (19)	73.5 [67.7; 78.8]	59.5 [55.1; 63.7]	0.67 [0.63; 0.71]	1.83	0.44
RCA					
766 (19)	73.0 [67.5; 77.0]	61.5 [56.9; 65.9]	0.67 [0.62; 0.71]	1.92	0.43
Supplied territory					
LM					
152 (4)	35.0 [23.1; 48.4]	75.8 [65.7; 84.2]	0.55 [0.46; 0.65]	1.58	0.83
Distal					
480(12)	72.7 [65.8; 79.0]	57.7 [51.8; 63.4]	0.65 [0.60; 0.70]	1.71	0.46
Lesion length					
Short (≤12 mm)					
1364 (33)	60.9 [56.7; 65.0]	68.5 [65.2; 71.7]	0.65 [0.62; 0.68]	1.98	0.56
Long (≥20 mm)					
327 (8)	66.8 [59.9; 73.3]	60.8 [51.7; 69.4]	0.64 [0.58; 0.70]	1.68	0.55
Vessel size by tertiles of reference diameter					
Small					
1363 (33)	53.1 [49.4; 56.7]	72.7 [69.0; 76.2]	0.63 [0.60; 0.66]	1.98	0.64
Intermediate					
1406 (34)	64.2 [60.3; 68.0]	67.8 [64.3; 71.2]	0.66 [0.63; 0.69]	2.09	0.52
Large					
1294 (32)	69.3 [65.1; 73.3]	61.9 [58.5; 65.1]	0.66 [0.63; 0.69]	1.78	0.47
B - Diameter stenosis cut-off value is set to 70%					
Categories n (%)	**Sensitivity** % [95% CI]	**Specificity** % [95% CI]	**Accuracy** AUC [95% CI]	**LR+**	**LR-**
Localization					
Overall					
4086 (100)	12.6 [11.2; 14.2]	97.9 [97.2; 98.4]	0.55 [0.48; 0.63]	6.75	0.89
LAD†					
1952 (48)	8.7 [7.1; 10.5]	98.8 [97.9; 99.4]	0.54 [0.51; 0.56]	7.77	0.92
LCx					
775 (19)	19.1 [14.5; 24.5]	96.5 [94.6; 97.9]	0.58 [0.53; 0.62]	5.57	0.84
RCA†					
766 (19)	21.3 [16.8; 26.4]	99.2 [97.8; 99.8]	0.60 [0.56; 0.65]	25.56	0.79
Supplied territory					
LM					
152 (4)	6.7 [1.9; 16.2]	98.9 [94.0; 100.0]	0.53 [0.43; 0.62]	6.31	0.94
Distal segments					
480 (12)	20.1 [14.6; 26.6]	95.5 [92.5; 97.6]	0.56 [0.52; 0.63]	4.58	0.83
Lesion length					
Short (≤12 mm)					
1364 (33)	14.5 [11.7; 17.7]	97.7 [96.4; 98.7]	0.56 [0.53; 0.59]	7.98	0.87
Long (≥20 mm)					
327 (8)	14.9 [10.3; 20.5]	96.8 [92.0; 99.1]	0.56 [0.50; 0.62]	4.70	0.88
Vessel size by tertiles of reference diameter					
Small††					
1363 (33)	9.0 [7.1; 11.3]	97.4 [95.8; 98.5]	0.53 [0.50; 0.56]	3.65	0.93
Intermediate					
1406 (34)	11.9 [9.5; 14.7]	98.6 [97.5; 99.3]	0.55 [0.52; 0.58]	9.63	0.89
Large††					
1294 (32)	18.8 [15.4; 22.5]	97.5 [96.2; 98.5]	0.58 [0.55; 0.61]	9.71	0.72

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Table 6 – Diagnostic accuracy of optimal angiographic cut-off values. Diagnostic performance of the optimal cut-off values in the overall population and in segments with large (left main) or small (distal segments) supplied myocardial territories.

DS - in different segments; n (%)						
Correlation r [95% CI]	**Cut-off** %	**Sensitivity** % [95% CI]	**Specificity** % [95% CI]	**Accuracy** AUC [95% CI]	**LR+**	**LR-**
Overall; 4086 (100)						
-0.38 [-0.41 ; -0.36]	51.2	57.9 [55.7 ; 60.2]	70.8 [68.8 ; 72.7]	0,69 [0.60 ; 0.78]	1.99	0.59
LM; 152 (4)						
-0.28 [-0.43 ; -0.13]	43.0	60.0 [46.5 ; 72.4]	68.5 [58.0 ; 77.8]	0.65 [0.56 ; 0.74]	1.96	0.57
Distal segments; 480 (12)						
-0.43 [-50 ; -0.35]	54.5	63.0 [55.6 ; 70.0]	70.5 [64.8 ; 75.6]	0.72 [0.67 ; 0.77]	2.12	0.52
MLD - in different segments; n (%)						
Correlation r [95% CI]	**Cut-off** mm	**Sensitivity** % [95% CI]	**Specificity** % [95% CI]	**Accuracy** AUC [95% CI]	**LR+**	**LR-**
Overall; 4086 (100)						
0.45 [0.42 ; 0.47]	1.49	75.2 [73.1 ; 77.1]	57.5 [55.4 ; 59.6]	0,72 [0.58 ; 0.86]	1.73	0.43
LM; 152 (4)						
0.32 [0.17 ; 0.46]	1.60	37.3 [25.0 ; 50.9]	90.1 [82.1 ; 95.4]	0.65 [0.56 ; 0.74]	3.30	0.69
Distal segments; 480 (12)						
0.48 [0.41 ; 0.55]	1.17	65.6 [58.2 ; 72.4]	73.4 [67.9 ; 78.4]	0.74 [0.69 ; 0.78]	2.40	0.46

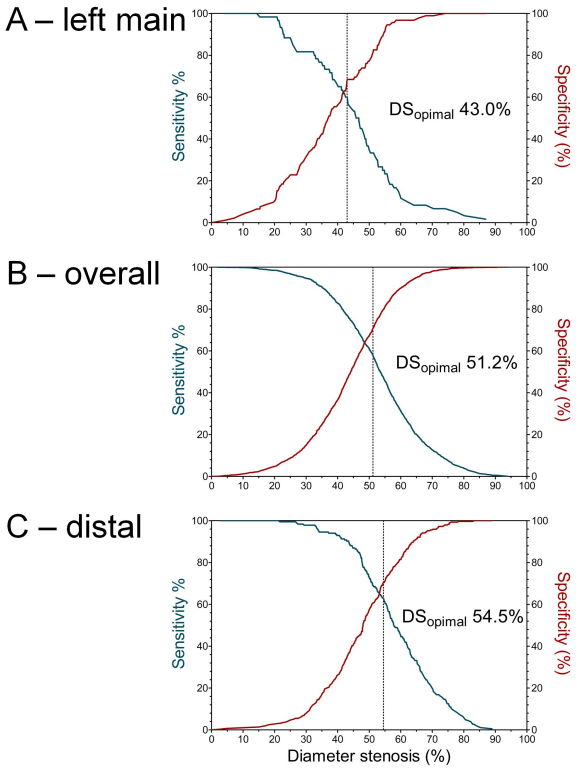

Figure 11 – Optimal cut-off values in different locations. Determination of the optimal cut-off values for diameter stenosis in different localization with different supplied myocardial territories, namely the left main stem (**Panel A**), the overall population (**Panel B**), and the distal segments (**Panel C**), as defined in the text above.

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6/2 – Results for FFR vs FFR_{myo} study

Data from 1.676 coronary artery stenoses in 1.235 patients were analyzed. Patients' clinical characteristics are summarized in **Table 7**. Indication for catheterization was heart failure with NYHA Class II-IV in 914 patients (74%), ischemic heart disease in 642 patients (52%) and concomitant valve heart disease in 593 patients (48%). Angiographic and hemodynamic data are summarized in **Table 8** and **Figure 12**.

Table 7 – Clinical characteristics and presentation	
Clinical characteristics	n = 1 235
Age (mean ± SD)	70.8 ± 9.8
Male gender (n, %)	796 (65)
Hypertension (n, %)	624 (51)
Hypercholesterolemia (n, %)	645 (52)
Diabetes mellitus (n, %)	256 (21)
Smoking (n, %)	441 (36)
Body-mass-index (mean ± SD)	26.5 ± 4.6
Clinical presentation	
NYHA II-IV functional status (n, %)	914 (74)
Ischemic heart disease (n, %)	642 (52)
Valvular heart disease (n, %)	593 (48)
Diastolic heart failure (n, %)	358 (29)
Systolic heart failure (n, %)	420 (34)

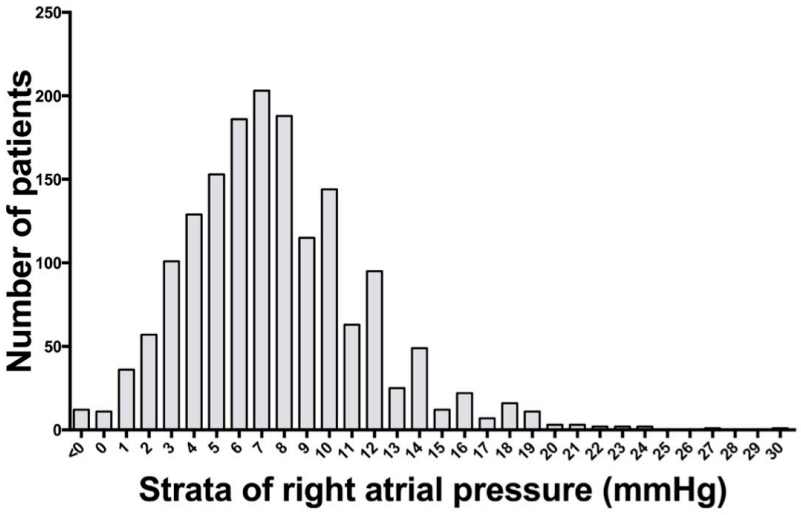

Figure 12 – Distribution of right atrial pressure values within included population.

Average FFR value was 0.85 (0.78; 0.91), while average FFR_{myo} was 0.83 (IQR 0.76; 0.90). Correlation and agreement between the two parameters were excellent (r^2=0.987; slope 1.096±0.003). The median difference between FFR and FFR_{myo} was 0.01 (0.01; 0.02). (**Figure 13**)

Table 8 – Angiographic characteristics. Hemodynamic parameters are indicated on patient level. Angiographic parameters are indicated on lesion level. Categorical variables are expressed as count (percentage). Continuous variables are expressed as mean ± standard deviation or median (interquartile ratio), as appropriate. FFR is fractional flow reserve. FFR_{myo} is myocardial fractional flow reserve.

Hemodynamic characteristics	n = 1 235
Left ventricular ejection fraction; %	61 ± 18
Patients with EF < 45%; n (%)	261 (21)
Mean arterial pressure; mmHg	90 (79 ; 100)
Left ventricular end-diastolic pressure; mmHg	17 (12 ; 22)
Pulmonary capillary wedge pressure; mmHg	16 (12 ; 22)
Right atrial mean pressure; mmHg	7 (5 ; 10); max: 27
Angiographic characteristics	n = 1 676
Lesion location:	
Left main stem, n (%)	134 (8)
Left anterior descending, n (%)	955 (57)
Left circumflex, n (%)	318 (19)
Right coronary artery, n (%)	269 (16)
Percent diameter stenosis, %	41 ± 18
FFR	0.85 (0.78; 0.91)
FFR_{myo}	0.83 (0.76; 0.90)

6/2.1 Relationship between FFR and FFR_{myo}

In patients, having normal right atrial pressure (P_{ra} ≤5mmHg) median difference between FFR and FFR_{myo} was minimal: 0.01 (0.00; 0.01). When grouping the patients

into tertiles of P_{ra}, a significant increase was observed in the difference between FFR and FFR_{myo} over the three groups [0.01 (0.00; 0.01) vs. 0.01 (0.01; 0.02) vs. 0.02 (0.01; 0.03), respectively; p<0.001]. (**Figure 14**)

The median difference between FFR and FFR_{myo} in lesions with FFR above 0.80 was 0.01 (0.00; 0.01). Out of 1146 stenoses with FFR above 0.80, none had an FFR_{myo} equal to or below 0.75; and 110 (9%) stenoses had an FFR_{myo} equal to or below 0.80. In the latter group the difference between FFR and FFR_{myo} was 0.02 (0.02; 0.03), yet with P_{ra} significantly higher than in the overall population [9 (7; 12) mmHg; p<0.001]. Receiver

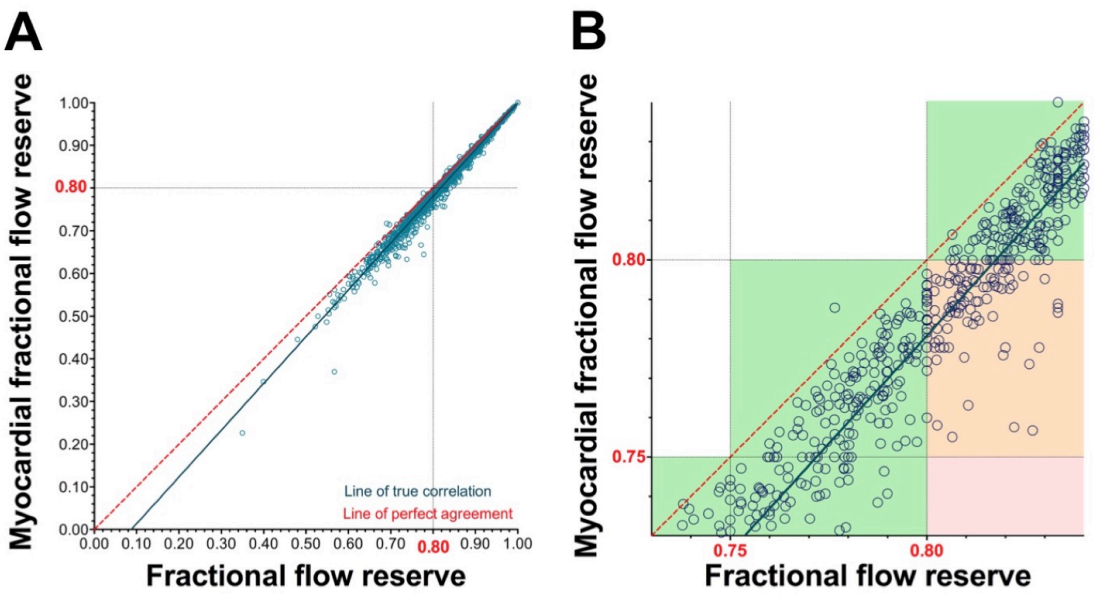

Figure 13 – Correlation between FFR and FFR_{myo}. Panel A depicts the correlation and agreement between FFR and FFR_{myo} in the overall population. Note, there is minimal deviation between FFR and FFR_{myo} at the lower third of the entire range of FFR 0 to 1. **Panel B** depicts that despite the deviation between FFR and FFR_{myo}, the vast majority of the measurements have clinical agreement (green areas), a minor portion of FFR above 0.80 turns to an FFR_{myo} below or equal to 0.80 (orange area) and no FFR above 0.80 turns to FFR_{myo} below or equal to 0.75 (red area).

operator characteristic analysis showed that 0.80 FFR value has 83% sensitivity and 100% specificity in predicting an FFR_{myo} below or equal to 0.80. Diagnostic accuracy expressed as area under the curve was 0.913 (95% confidence interval: 0.896 to 0.931). The best cut-off value of FFR for predicting an FFR_{myo} below or equal to 0.80 was found to be 0.82 with 96% sensitivity and 97% specificity.

These findings remained unchanged when accounting for multiple lesions for some patients or when selecting at most one lesion per patient in random fashion justifying the irrelevance of any clustering effect.

6/2.2 Effect of varied right atrial pressure values

We assessed 2 models of possible impact of P_{ra} on FFR measurements based on the available datasets:

(1) In the first model, FFR_{myo} was calculated for the same patient population, applying 3 fixed values of potential P_{ra}: i.e. 5 mmHg, 10 mmHg and 20 mmHg. A significant increase was observed in the difference between FFR and FFR_{myo} over the three values, however this remained remarkably low [0.01 (0.01; 0.01) vs. 0.02 (0.01; 0.03) vs. 0.04

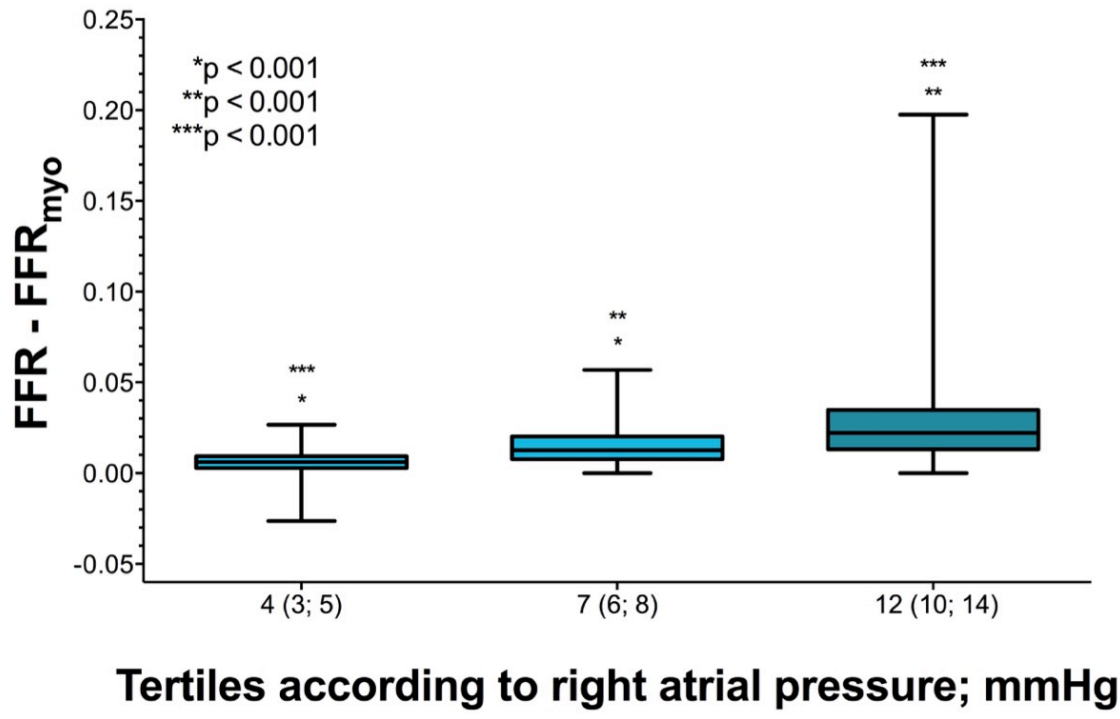

Figure 14 – Differences between FFR and FFR_{myo}. Significant constant increase was observed in the difference between FFR and FFR_{myo} over the tertiles by right atrial pressure. However, even in the highest tertile the mean difference remained clinically minimal.

(0.03; 0.07), respectively; p<0.001]. In the 5 and 10 mmHg groups, values of FFR>0.80 never turned to an FFR_{myo}≤0.75; while in the 20 mmHg group, this occurred in 4% of the cases. In addition, no FFR values above 0.82, above 0.83 and above 0.87 would have turned to FFR_{myo} equal or below 0.80 in the three groups, respectively (**Figure 15**). (2) In the second model, we investigated on the same population what P_{ra} value could have a relevant impact on the following threshold values of FFR: (i) FFR above 0.80 and FFR_{myo} equal or below 0.80, or (ii) FFR above 0.80 and FFR_{myo} equal or below 0.75. With normal P_{ra}, FFR above 0.80 never turns to FFR_{myo} equal or below 0.80 with FFR higher than 0.82. With normal P_{ra} (≤ 5 mmHg), FFR above 0.80 never turns to FFR_{myo} equal or below 0.75 in any case. The latter might only occur in case FFR is close to the cut-off value of 0.80, or P_{ra} is particularly (even non-physiological) high.

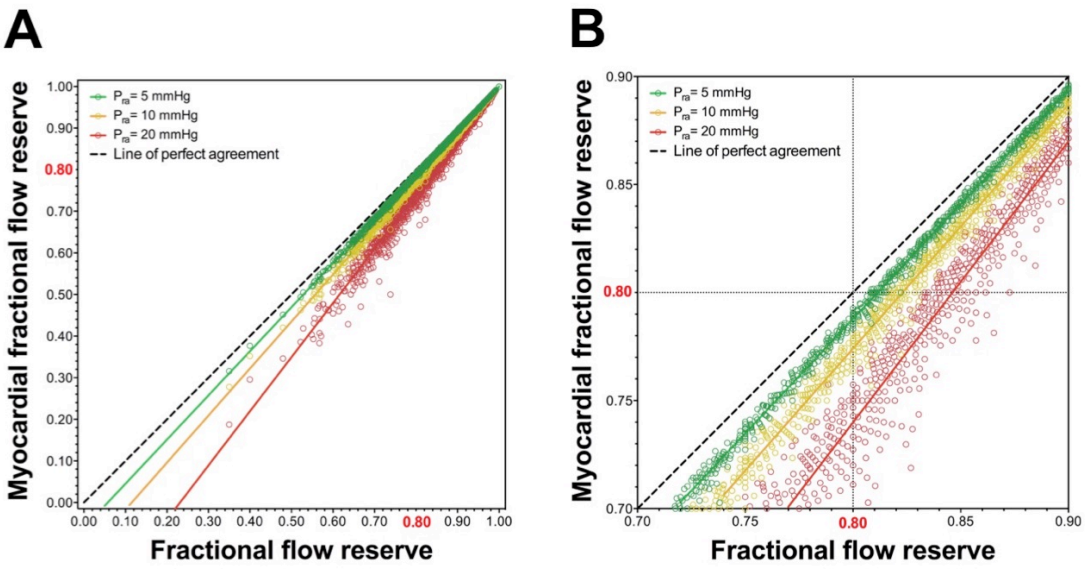

Figure 15 – Simulation of relation between FFR and FFR_{myo} at different right atrial pressure values. Figure indicates FFR and related FFR_{myo} values, calculated by applying three different potential P_{ra} values, namely 5 mmHg, 10 mmHg and 20 mmHg. Significant increase was observed in the deviance from agreement between FFR and FFR_{myo} over the three groups. (**Panel A**) No FFR values above 0.82, above 0.83 and above 0.87 would have turned to FFR_{myo} equal or below 0.80 in the 5 mmHg, 10 mmHg and 20 mmHg groups, respectively. (**Panel B**)

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6/3 – Results for Dose-response study

Characteristics of the 30 subjects are summarized in **Table 9**. One subject received only 3 doses of intracoronary adenosine (4, 12, and 20 μg), and so was excluded from the ANOVA dose-response analysis but included in all other analyses. While all vessels were free of any visible stenosis, the CFR varied from 1.42 to 4.88. The baseline flow velocity was higher in patients with a low CFR than in patients with a high CFR. (29±11 cm/s versus 16±7 cm/s; p<0.001). Hyperemic flow velocity was similar in both groups (61±26 cm/s versus 55±17 cm/s; p=0.41).

Table 9. Patients characteristics and medication

Patient demographics	**(n=30)**
Age	65±11 years
Male	26 (87%)
Body weight	77±15 kg
Height	171±9 cm
Hypertension	17 (59%)
Hypercholesterolemia	18 (62%)
Diabetes mellitus	4 (14%)
Smoking	7 (24%)
Prior PCI	10 (34%)
Prior myocardial infarction	1 (3%)
Medication (n=30)	
Aspirin	24 (80%)
Clopidogrel	9 (30%)
Ticagrelor	6 (20%)
Statin	23 (77%)
Beta blocker	10 (33%)
Calcium channel inhibitors	8 (27%)
Inhibitors angiotensin converting enzyme	10 (33%)
Angiotensin II receptor blocker	6 (20%)
Nitroglycerin	1 (3%)
Oral antidiabetic drugs	1 (3%)
Insulin	3 (10%)

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6/3.1 Dose-response analysis

Figure 16 summarizes the dose-response relationships and also displays the incidence of high-grade AV-block for each dose of intracoronary adenosine. One subject received only 20 μg because the quality of the flow velocity signal deteriorated and could not be restored. All episodes of AV-block were transient and none required specific treatment. However, episodes of transient AV-block occurred at doses higher than 100 μg, precluding the administration of higher amounts than 300 μg of intracoronary adenosine in 5 (17%) patients.

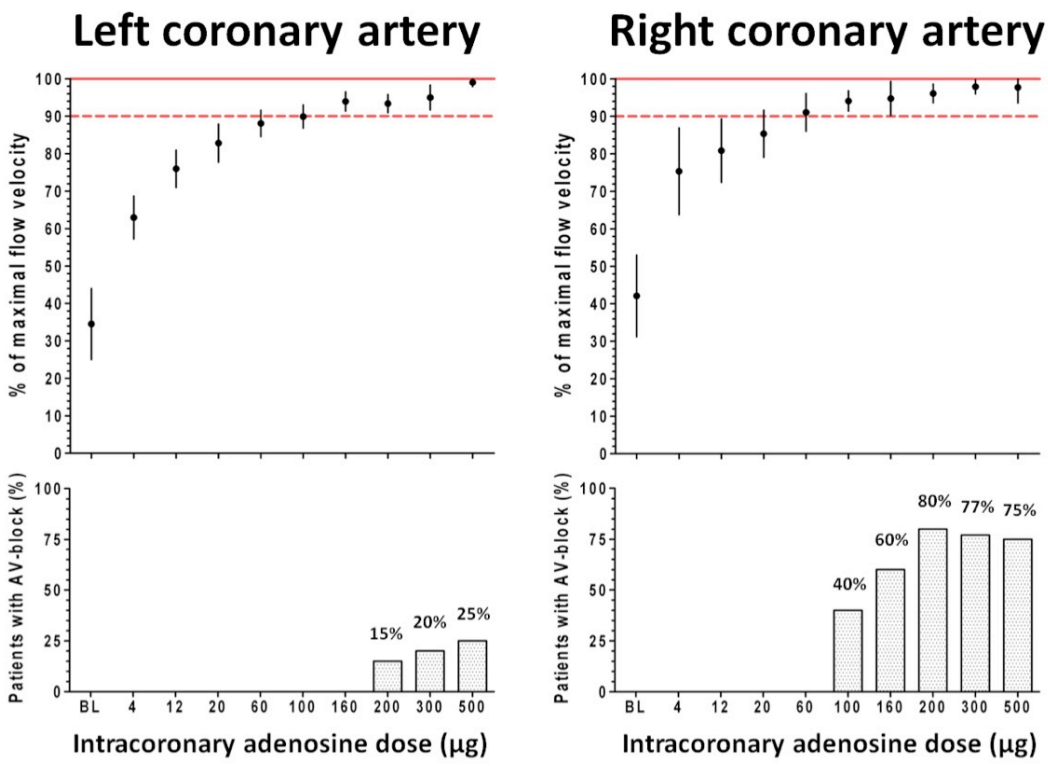

Figure 16 – Dose-response analysis and corresponding occurrence of high-grade AV-block. *Top panels*: dose-response data for the right (RCA, left panel) and the left coronary arteries (LCA, right panel). The data are expressed as the percent of maximum for each patient (Q/Q_{max}) at each dose of intracoronary adenosine. The error bars represent the 95% confidence intervals for each value. The p-values from the subsequent Tukey paired comparisons are given in **Table 10**. *Lower panels*: the bars represent the percent of patients in whom high-grade AV-block occurred with that dose of adenosine.

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Significant differences in normalized flow velocity (Q/Q_{max}) existed via mixed effects ANOVA analysis for all vessels together and for the right coronary artery (RCA) and left coronary arteries (LCA) separately ($p<0.001$ for all). **Table 10** displays the p-values from the subsequent Tukey paired comparisons on a per-vessel basis. For the RCA,Q/Q_{max} did not increase significantly at any higher dose than 60 μg. For the LCA and all vessels together, Q/Q_{max} did not increase significantly at any higher dose than 160 μg.

Table 10 – Dose-response analysis for normalized maximal flow velocity (Q/Q_{max})
Analysis is showing pairwise p-values (ANOVA then Tukey all-pair comparison) comparing various intracoronary adenosine doses (from baseline to 500 μg) for the right (RCA) and left coronary arteries (LCA).

RCA	4 μg	12 μg	20 μg	60 μg	100 μg	160 μg	200 μg	300 μg	500 μg
Baseline	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
4 μg		0.74	0.041	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
12 μg			0.94	0.055	0.002	0.004	<0.001	<0.001	0.008
20 μg				0.71	0.13	0.19	0.019	0.001	0.15
60 μg					0.99	1.00	0.83	0.35	0.93
100 μg						1.00	1.00	0.93	1.00
160 μg							1.00	0.91	1.00
200 μg								1.00	1.00
300 μg									1.00

LCA	4 μg	12 μg	20 μg	60 μg	100 μg	160 μg	200 μg	300 μg	500 μg
Baseline	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
4 μg		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
12 μg			0.007	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
20 μg				0.23	0.010	<0.001	<0.001	<0.001	<0.001
60 μg					0.98	0.11	0.28	0.004	<0.001
100 μg						0.77	0.95	0.15	0.011
160 μg							1.00	0.99	0.68
200 μg								0.91	0.38
300 μg									1.00

Figure 17 shows the mean duration of plateau hyperemia, the time needed to return to baseline, as well as the effect on heart rate and blood pressure. For a bolus of 100 μg in the RCA, plateau hyperemia lasted 12±13 s. For a bolus of 200 μg in the LCA, plateau hyperemia lasted 21±6 s. The time needed for the flow velocity to return to baseline increased progressively with the intracoronary adenosine dose. In 10% of patients the flow velocity did not return to baseline within 2 minutes after at least one intracoronary adenosine administration.

While there was no significant change in heart rate among doses of intracoronary adenosine (ANOVA p=0.48), mean arterial pressure was altered (ANOVA p=0.001). Tukey all-pair comparison of mean arterial pressure showed significant decreases with all doses of intracoronary adenosine compared to baseline conditions (all $p<0.05$)

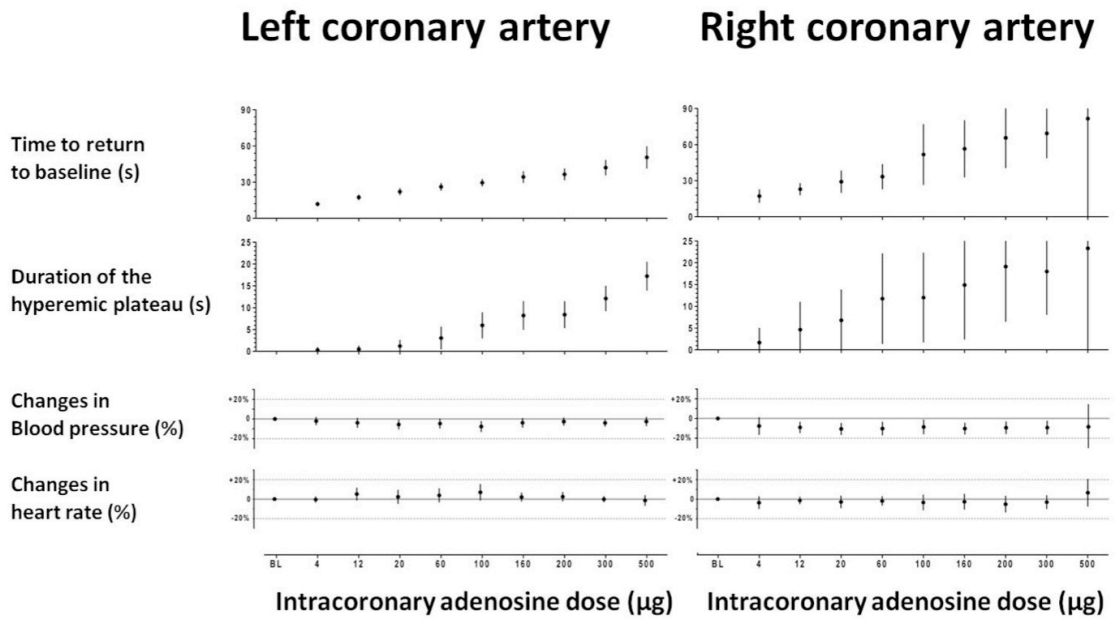

Figure 17 – Dose-response summary data. Figure depicts the summary for duration of hyperemic plateau (seconds), time needed to return to baseline flow velocity value (seconds), systemic blood pressure (mmHg), and heart rate (bpm).

except for 4 μg (p=0.24), but not between adenosine doses (all $p>0.60$). Mean arterial pressure decreased with intracoronary adenosine by about 6-7% from baseline based on the mixed effects model.

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6/3.2 Effect of blood, saline and contrast medium

Doppler flow velocity varied among 8 mL intracoronary boluses of arterial blood, saline and contrast ($p<0.001$ by ANOVA), and all pairwise comparisons were significant ($p<0.001$ for blood and contrast; $p=0.041$ for saline and blood; $p=0.013$ for saline and contrast). As shown in **Figure 18**, contrast increased Doppler flow velocity the most (+38±52% over blood, $p<0.001$ by paired t-test; +17±28% over saline, $p=0.019$) and saline was superior to blood (+21±43%, $p=0.008$). Flow velocity after contrast medium reached 65±36% of the value reached after 200 μg of adenosine. Heart rate and mean arterial pressure did not change significantly after administration of arterial blood, saline, or contrast ($p=0.19$ for pressure, $p=0.37$ for heart rate by ANOVA). An 8 mL bolus injection of 200 μg adenosine mixed with contrast medium showed no hyperemic synergy, neither prolongation of hyperemia ($p=0.14$ for interaction by ANOVA).

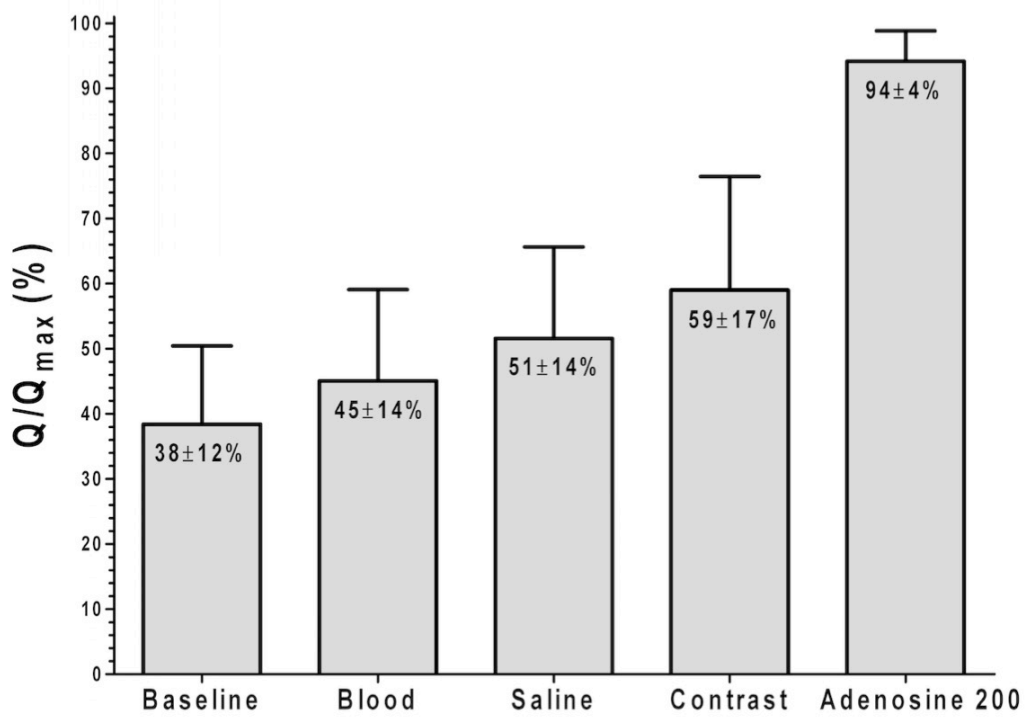

Figure 18 – Application of other, potentially hyperemic media. Effect on coronary Doppler flow velocity from the administration of an 8 mL bolus of arterial blood at body temperature, saline at room temperature, and contrast medium at room temperature Data are given as average±SD of the percentage (%) of maximum flow velocity for each patient (Q/Q_{max}).

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6/3.3 Dose-response model and impact on FFR

Figure 19 (left panel) shows both the raw Q/Q_{max} data and the best-fit models for each artery. Greater flow increases were observed in the RCA than the LCA for the same intracoronary adenosine dose. No important differences existed between model parameters from a fixed effects model (RCA k = 2.84 μg, 95% confidence interval [CI] 2.05 to 3.93, and offset = 2.21 μg, 95%CI 1.40 to 3.56; LCA k = 3.95 μg, 95%CI 3.33 to 4.68, and offset = 2.46 μg, 95%CI 1.89 to 3.22) and a mixed effects model (RCA k = 3.05 μg, 95%CI 1.81 to 4.29, and offset = 1.94 μg, 95%CI 1.43 to 2.46; LCA k = 4.07 μg, 95%CI 4.02 to 4.11, and offset = 2.26 μg, 95%CI 2.22 to 2.29). Based on these

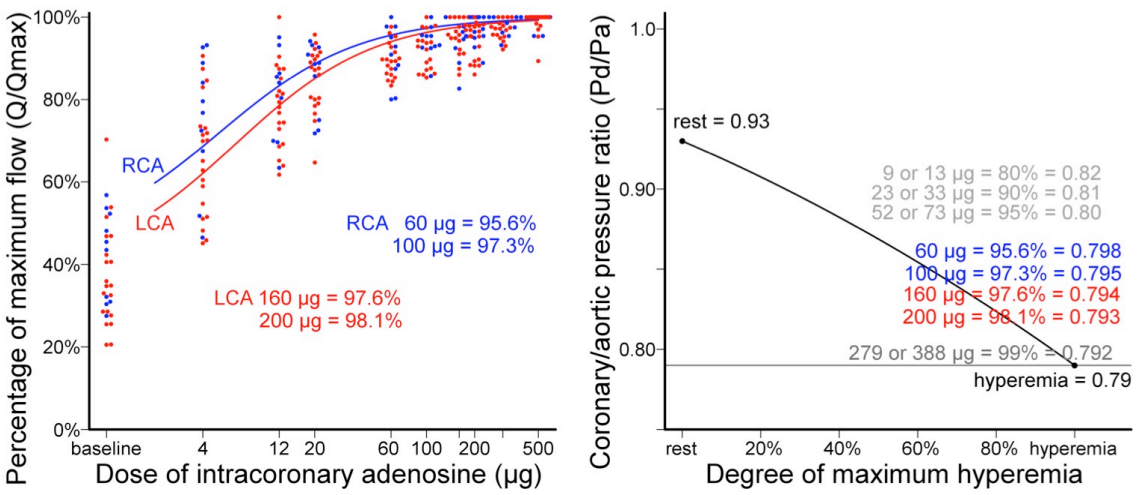

Figure 19 – Dose-response model and its effect on FFR. The left panel plots the percentage of maximum flow velocity for each patient (Q/Q_{max}) as solid dots with their best-fit line (red for the left coronary artery [LCA] and blue for the right coronary artery [RCA]) as a function of the intracoronary dose on a logarithmic x-axis (baseline placed at 1 μg). Note that overlap occurs among points at high doses. The right panel translates the dose-response curve into the observed FFR as a function of adenosine dose (RCA or LCA) for a typical lesion. This theoretical model shows that at 60 to 100 μg in the RCA and at 160 to 200 μg (colors match left panel) the observed FFR is within 0.01 of its minimum value. In addition, at dosages above 23 μg, the observed FFR is within 0.02 of its minimal value.

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dose-response models, intracoronary adenosine reaches 80% of maximum hyperemia at 9 or 13 μg (RCA versus LCA), 90% of maximum hyperemia at 23 or 33 μg, 95% of maximum hyperemia at 52 or 73μg, and 99% of maximum hyperemia at 279 or 388 μg. **Figure 19** (right panel) combines this dose-response relationship with a physiologic model linking flow increase to the observed FFR. At 0% hyperemia (baseline conditions) P_d/P_a=0.93, while at 100% hyperemia (maximum) FFR=0.79. Intracoronary adenosine doses between 60 and 200 μg provide an FFR within 0.01 deviation of the value at 100% hyperemia.

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7. Discussion

Since its first introduction [41-44;58] the concept of FFR and the potential clinical benefits, associated to its use for guiding revascularization strategies have been supported by powerful outcome data, derived from large multi-centric randomized clinical trials [54;65-67;71;72;75] and registries. [68;69;74;83-90] Accordingly, FFR became the standard of reference to define the ischemic potential of a given coronary stenosis and to guide revascularization decisions, when non-invasive ischemia tests are not available or inconclusive. [45]

As, despite clear recommendations, most of the decisions in the catheterization laboratory are still based on pure angiographic metrics, the primary goal of this work was the understanding of the value of angiography, in terms of determining true functional stenosis significance. Additionally the analysis allowed us to have an insight, even if speculative, into the potential clinical consequences. Therefore, the first part of the present work was designed to evaluate the relation between morphology and function, namely QCA-based stenosis severity and ischemic potential by FFR in a large unselected patient cohort.

Present work reports data from the largest patient population in the topic so far. Our results emphasize that reliance on the angiogram needs to be modified by physiologic measures of severity for a wide range of intermediate stenoses. Explaining the results of randomized trials, showing outcome benefit associated with FFR-guidance, we found that as much as one third of the decisions based angiographic metrics are discordant with the FFR. Note, in contrast with previous work [117] present data was based on quantitative assessment of the angiogram.

Traditionally, management of CAD has been based on the pure angiographic threshold of 50% DS. [33] Validation of patient risk-stratification, non-invasive testing and studies of revascularization have used this criterion as standard of reference. [11-26] However, the 50% DS threshold was derived from animal experiments, which showed that hyperemic coronary flow reserve started to decline below 4.0 when DS was ≥50% or below 3.0 when DS was ≥70%DS. Note, these fluid dynamic endpoints were not linked to ischemia, left ventricular function or clinical outcomes. In addition, these data were interrogated in animal experiments, investigating ‘healthy, young, anesthetized’ dogs, questionably representing our routine patient population. [3;4] In humans with

proven atherosclerosis, a similar relationship between DS and myocardial blood flow has been shown although this correlation is substantially diminished by a very large scatter [62;63] Again, no relation to ischemia, left ventricular function or clinical outcomes has been shown.

In many early studies that have shaped our understanding of the relationship between CAD, revascularization and clinical outcome, the threshold of 70% DS has been used for assessing prognosis or accuracy of non-invasive imaging. [15;16;118] Present data show that increasing the threshold to 70% improved the specificity (i.e. will decrease the trend of coronary angiography to overestimate lesion severity, resulting potentially in unnecessary revascularization) but decreased sensitivity (i.e. will increase the number of stenoses underestimated by coronary angiography, resulting potentially in untreated risk left behind). Summed, increasing the threshold to 70% decreases the overall diagnostic performance of DS in predicting FFR≤0.80 as compared to 50% cut-off value. Both types of misclassifications may have important clinical consequences. Recent outcome studies have demonstrated that revascularization of non-significant stenoses can be safely deferred [54;65], and that the revascularization of non-significant lesions (*overtreatment*) is inappropriate with adverse procedural risk without offsetting benefit. [71;72] Conversely, denying revascularization to patients with hemodynamically significant stenoses (*undertreatment*) is detrimental. [66;67]

The present data also show that the optimal diagnostic threshold of DS is markedly lower in coronary segments supplying larger myocardial area than in segments supplying small myocardial area. At first glance, this phenomenon is surprising, since DS factors in myocardial mass to be perfused by a given segment. Seiler et al. showed that the normal coronary diameter (the denominator of DS) correlates linearly with myocardial mass. [119] In atherosclerotic vessels, this relation is flatter than in normal arteries. This observation might explain why a less severe DS is associated with a lower FFR in large versus small arteries. Reciprocally, a small artery may have higher FFR than a large artery for comparable anatomic stenosis, thereby indicating that FFR depends to some extent on the downstream mass. Practically, this finding implies that a LM stenosis may reach hemodynamic significance (FFR≤0.80) for a lesser degree of DS than a distal arterial segment. Thus, in the present data, the underestimation by the angiographic 50% cut-off was markedly more frequent among LM stenoses than in

distal segments. This hypotheses can be strengthened by the comparison of lesions in the proximal LAD, with large supplied myocardial territory, versus lesions in distal coronary segments, with limited supplied myocardial mass: categorizing the stenoses according to the strata of QCA we found that FFR values, belonging to lesions in the proximal LAD are consequently lower as compared to the same in distal coronary segments, emphasizing the importance of supplied myocardial mass. (**Figure 20**)

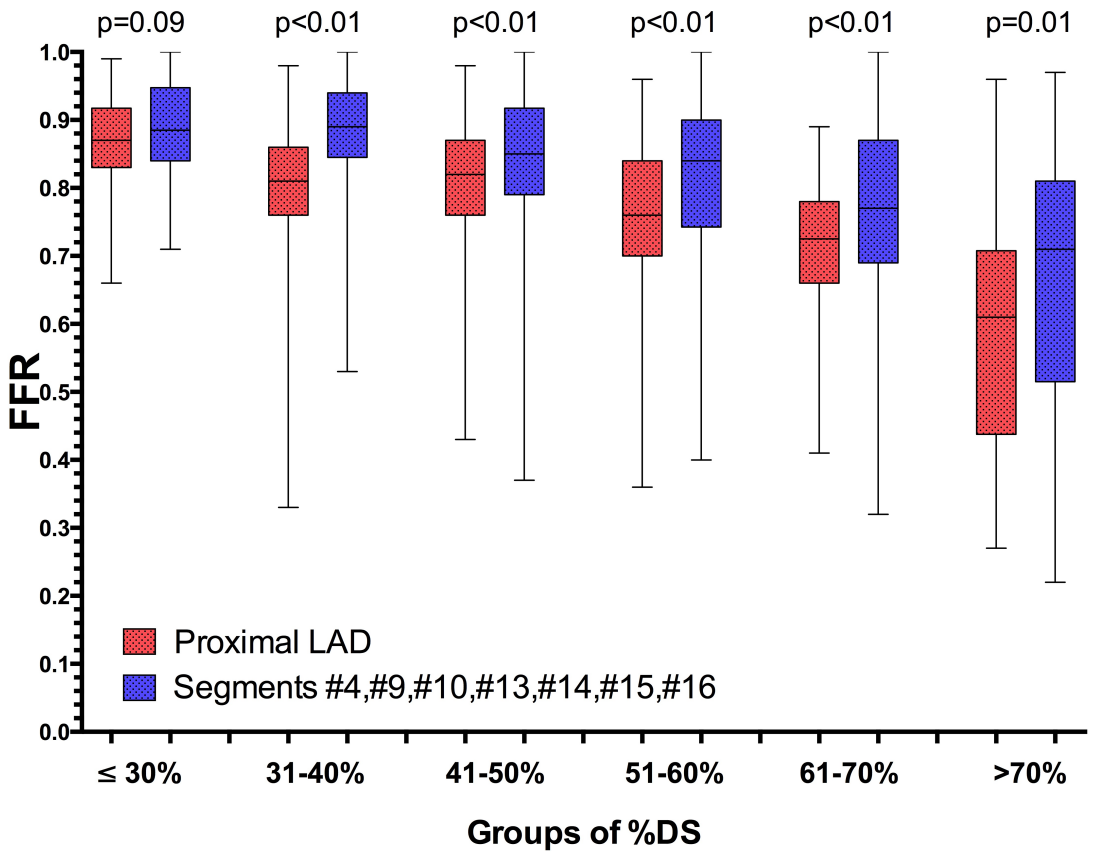

Figure 20 – FFR value according to strata of QCA. Comparison of lesions in the proximal LAD, with large supplied myocardial territory, versus lesions in distal coronary segments, with limited supplied myocardial mass. FFR values, belonging to lesions in the proximal LAD are consequently lower as compared to the same in distal coronary segments, emphasizing the importance of supplied myocardial mass.

Interestingly significant difference in diagnostic accuracy was found between males and females, and between diabetic and non-diabetic population. (**Table 4**) Explanation can be only speculative, as many factors, including technical limitations as well as

physiologic conditions may contribute to explain the poor correlation between angiographic indices and FFR, where the latter (including diffuse disease, impaired microvascular compartment, etc.) might be more pronounced in male and in diabetic population with higher risk of atherosclerosis. Additionally both QCA and FFR suffer inherent measurements uncertainties and imperfect repeatability that limit the expected correlation. The lack of standardization of FFR measurements and recordings, especially in the beginning of the experience, may account for some scatter, however recent data have shown that not even a core lab analysis can eliminate the (otherwise minimal) intrinsic variability of FFR. [124] More relevant issue can be the technical and methodical limitations of QCA. Angiographic border detection can be difficult in patients with diffuse CAD, especially when calcifications are present. Overlap with other arterial segments, foreshortening of the segment, bifurcation and ostial stenoses, and expansive vessel remodeling may further complicate the calculation of DS. The MLD, which consists of one single measurement, should be less influenced by technical inaccuracies. Yet, this advantage is offset by the fact that the physiological impact of MLD is also related to the downstream myocardial mass to be perfused. Mass dependence may explain the threefold difference between the optimal cut-off value of cross sectional area between the LM and the distal segments. Combining DS and MLD might confer more precision to angiography as suggested by Fischer et al. who found that no patient with stenosis <60% or minimal luminal diameter >1.4 mm had FFR <0.75. [120] However this ‘upgrade’ of angiographic evaluations is still suffering all the limitations of a solely anatomic approach: All techniques aiming at quantifying a single luminal measurement (DS, MLD or cross sectional area) face the same intrinsic limitation of being segment-related, not considering the status in distality (i.e. collaterals, supplied tissue, etc.) For all reasons noted here, coronary anatomy alone – even with the highest resolution and a hypothetical perfect repeatability – will never be sufficient to predict physiological behavior of a single stenosis. [121;122]

The main unknowns are the myocardial mass depending from the stenotic segment and the microvascular function. Both will determine maximal myocardial blood flow, which is essentially measured by FFR. In contrast, FFR is a flow index. As such, its value is influenced and integrates hyperemic flow, which itself depends on stenosis severity, myocardial mass and its microvascular function. Since mass and microvascular function

are not likely to change in a given patients before and after revascularization, FFR indicates to what extent hyperemic myocardial flow will increase after PCI (i.e. normalization of the epicardial resistance). Therefore, FFR can be considered specific to the epicardial segment, which constitutes the basis of its clinical utility. From the previous discussion, it can be hypothesized that measurements of absolute myocardial blood flow is the major missing link to explain the fundamental discordance between angiography and FFR (the red and yellow quadrants on **Figure 9**).

Naturally, this analysis has a couple of limitations to be acknowledged. The majority of the cases in the dataset were chosen based on the operators discretion, when the anatomy could not give the clinical answers, and was the indication for FFR. Therefore some 'referral bias' cannot be avoided and the conclusions should be limited to intermediate stenoses by visual assessment (i.e. approximately between 30 and 90% DS). Second, the data were collected over a long period of time and database analysis was performed retrospectively. This might have affected the accuracy of the angiographic measurements, as several technicians performed the measurements. Third, QCA analysis was not done by a dedicated core lab but by highly experienced technicians. Fourth, angiographic analysis was performed in one, the most severe projection only. Since coronary stenoses are asymmetrical, it is likely that a three-dimensional reconstruction of the artery would provide better correlations. [123]

Summarizing the findings for the first main topic of this work, the present data confirm that in comparison with lesion level ischemic potential, expressed by FFR, coronary angiography underestimates or overestimates physiologic stenosis severity in a large proportion of angiographically intermediate stenoses that may trigger inappropriate decisions about revascularization. Inappropriate decisions can be translated to over- or undertreatment of patients, with all potential clinical, economical and logistical consequences. Data suggest that the discordance between morphology and function relates to differences in supplied viable myocardial territory, to differences in microvascular function, but also to technical limitations of angiographic technology, such as 2- and 3-dimensional resolution.

The physiologic and clinical value of the method can be understood when realizing that although FFR is calculated from the ratio of two pressures, it expresses the relation of

two flows. As explained in details above, the standard formula for FFR is simplified by excluding P_{ra}, being considered negligibly low as compared to arterial values. In this work we investigated on the largest patient population so far, affected by various degree of heart failure of different etiologies, whether incorporating the value of right atrial pressure into the formula has any clinical impact on FFR measurement. Although these patients had a P_{ra} often markedly above the normal range, the correlation and the agreement between measured FFR and calculated FFR_{myo} was still excellent, with a difference as minimal as 0.01. In a small fraction of patients (9%) the FFR value went from above 0.80 to an FFR_{myo} value equal to or below 0.80, but still with an individual difference of no more than 0.03. In these patients, the mean P_{ra} was 9 (7; 12) mmHg. More importantly in no case an FFR value above 0.80 turned to an FFR_{myo} below 0.75. Interestingly this observed difference between the two values is still within the range of test-retest repeatability of FFR measurements. [124] These data were confirmed in the theoretical models showing even extreme right atrial pressure (≥ 20 mmHg) to marginally impact the FFR - FFR_{myo} relation.

From a pure clinical and pragmatic perspective, it might be observed that in patients with right atrial pressure above 20 mmHg and, presumably, signs of right heart failure, the fact to knowing whether the FFR value is just above or just below the ischemic threshold will likely have little impact on their prognosis or on the clinical decision-making process.

The present study was conducted in patients requiring left and right heart catheterization for clinical reasons. This might have induced a selection bias as far as the average level of P_{ra} is concerned. In patients without clinical indication for a left and right catheterization, it is likely that the average P_{ra} would have been markedly lower. This would have lead to an even smaller difference between FFR and FFR_{myo}. However, even with this large pool of patients we could find only a minority with extreme right atrial pressure far above 20 mmHg. This might be suggestive of the lack of need or indication to perform FFR assessment in a clinical setting of very elevated filling pressures like for example in case of cardiogenic shock or acute heart failure.

In addition, the lesions analyzed in the present study were not equally distributed over the entire range of FFR values, but rather clustered in the intermediate range (between 0.70 and 0.90), reflecting the clinical indication of FFR measurements in daily practice.

The more severe the lesion, the larger the absolute difference between FFR and FFR_{myo} for a given P_{ra} value, yet – simultaneously - the more trivial the clinical consequences of this difference. Whether the FFR is 0.50 rather than 0.55 has no clinical consequences.
In 9% of the patients, FFR value went from above 0.80 to an FFR_{myo} value equal or below 0.80. One might say, according to the guidelines [45], these patients would have been considered for revascularization, had right atrial pressure been implemented in the calculation of FFR. But please note, large clinical outcome data supporting FFR-guided management, are derived from studies using FFR and not FFR_{myo}. This slight underestimation of the true FFR_{myo} can perfectly be acceptable, given the expected limited clinical impact on one side, and the increased adjunctive complexity of FFR assessment when right atrial pressure would be systematically assessed in current busy cathlab activities. Finally, patient's clinical outcome was beyond the scope of this investigation and was therefore not obtained.
Summarizing, our findings indicate that accounting for actual value of right atrial pressure induces only minimal differences in the calculations of FFR, on average within the limits of the test-retest repeatability. In addition, these differences have negligible clinical consequences even in patients with elevated right atrial pressure. Our data confirm that there is no reason and no need for reconsidering the standards of practice for FFR measurement, but right atrial pressure can be indeed neglected in day-by-day FFR assessment, in keeping with clinical outcome data.

As shown, incorporation of right atrial pressure in the formula of FFR has only minimal impact and rather no clinical relevance. But what is indeed crucial is the induction of reliable maximal hyperemia. Early seminal animal experiments have shown, as well as described in the methodology paper of FFR, coronary pressure becomes linearly correlated with the coronary flow only during maximal hyperemia, namely when all autoregulatory mechanisms are 'switched off'. [41-44;47;58] Still there are attempts to both, either 'over-simplifying' the method by getting rid of the hyperemic stimulus by introducing so called resting indices [125], or to administer extreme doses of adenosine in order to 'further increase' the level of hyperemia. [50] However, it was fundamentally proven that application of any non-maximal hyperemic index results in significant reduction of diagnostic accuracy. [115;124;126] In this work we aimed to

investigate this scientific and practical problem from the other direction, namely to clarify, what is the optimal dose of adenosine to induce maximal achievable hyperemia. The analysis allowed us to evaluate, whether any dose-reduction could be done without loss of diagnostic accuracy or on the other hand, whether any dose-increase would provide with any diagnostic benefit. The present dose-response study of intracoronary adenosine on intracoronary Doppler flow velocity suggests that the optimal bolus to induce maximal hyperemia consistently, reliably and safely is 60-100 μg for the RCA and 160-200 μg for the LCA. While sequential doses above 60 μg for the RCA and 160 μg for the LCA showed no statistically significant further increase in flow (see **Table 8**), the entire dose-response continuum (see **Figures 16** and **19**) demonstrates a reduction in inter-individual variability around 100 to 200 μg, respectively. Additionally, an undefined proportion of adenosine can potentially spill into the aorta during intracoronary administration, further implying the need for a safety margin.

Notably, we observed an increased incidence of AV-block at high doses (see **Figure 16**). The occurrence of a transient AV-block creates artifact on the tracings. Albeit always transient, such episodes of AV-block are disruptive during a catheterization procedure and might cloud the accuracy of the measurements, thus arguing for modest yet sufficient doses. Therefore, based on our findings a dose of 100 μg for the RCA and 200 μg for the LCA is recommended, balancing hyperemia versus side effects.

The present data confirm that the administration of the recommended intracoronary adenosine does not induce any patients discomfort or any clinically significant changes in heart rate, blood pressure, or ST-T segment. [127] Even at low doses (4 and 12 μg), a marked increase in flow velocity was observed in all patients eliminating the possibility of any ‘resistance to adenosine’. The plateau phase of maximal hyperemia at suggested optimal doses averaged for the RCA and the LCA are respectively 12±13 and 21±6 seconds, long enough to make accurate measurements, but too short to perform pull back recordings. The time to return to baseline were 38±20 seconds for the RCA and 77±10 seconds for the LCA, after administration of 100 and 200 μg respectively. These durations of action permit reliable yet quickly repeated measurements.

Akin for FFR measurements, we did not flush the dead space in order to avoid the ‘flush artifact’ on the aortic pressure tracings. This implies that the actual dosage of

adenosine reaching the coronary ostium is approximately 15% lower than the amount leaving the syringe.

Because of the relatively short-lasting action of intracoronary adenosine, we recommend to record at least 10 beats at rest, followed by a short lasting bolus injection, immediate reconnection of the aortic pressure signal and a total duration of the recording of 60 seconds. Such recording allows complete view on baseline gradient and hyperemic response, providing with highest quality of archived data. This recording should then be repeated in the exactly same manner and stored. This standardization of the recordings is important to allow their interpretation and review. With increasing dosages we also observed a prolongation of the time needed to return to baseline. At higher dosages, coronary blood flow velocity did not return to baseline despite waiting for several minutes. It may be speculated that repetitive episodes of hyperemia (and of ischemia) lead to an up-regulation of the adenosine receptors or of other mediators involved in the molecular pathways leading to microvascular dilatation. The maintenance of a higher flow after several episodes of hyperemia questions further the value of physiological lesion assessment at rest soon after coronary intervention without induction of maximal hyperemia.

Because of curvilinear relationships between intracoronary adenosine dose and Doppler flow velocity as shown in **Figure 16**, and between the degree of maximum hyperemia and P_d/P_a as known from fundamental stenosis physiology, the net effect produces clinically similar FFR values for even modest doses of intracoronary adenosine, as will be detailed next.

Recent work has determined that the test-retest repeatability of FFR has a standard deviation of approximately 0.02 [126] Thus FFR differences <0.02 as seen in **Figure 18** for adenosine doses above about 40 μg are smaller than the variability of the measurement itself. Interpreting the dose-response curve from this perspective, changes in flow response for intracoronary adenosine doses above 40 μg are smaller than the intrinsic variability of the FFR measurement. As such, while large studies might show a statistically significant difference in FFR for higher doses of intracoronary adenosine, test/retest repeatability indicates that these differences are not clinically significant.

On a related point, earlier work measured FFR using doses of intracoronary adenosine in the 30-60 μg (left) and 20-30 μg (right) range. For example, the pivotal DEFER trial

employed intracoronary adenosine in 42% of cases, delivering 20 μg (left coronary artery) and 15 μg (right coronary artery). [54;65] Our current dose-response relationship in **Figure 19** (left panel) clarifies that 15 μg achieves at least 80% and 35 μg at least 90% of maximum hyperemia. As translated by **Figure 19** (right panel), these levels of hyperemia would result in typical FFR measurements within 0.02-0.03 of higher doses. Correspondingly, DEFER found average FFR values using intracoronary adenosine that were larger than but still within 0.02 of intravenous adenosine, albeit in distinct patients (intracoronary versus intravenous adenosine: reference group 0.58 versus 0.56; performance group 0.88 versus 0.86; and deferral group 0.86 versus 0.87, all not statistically significant). In agreement with our current findings, these small differences in FFR were neither clinically nor statistically significant in DEFER.

By distinction, we systematically injected intracoronary adenosine in range from 4 μg up to 500 μg and employed a specific dose-response model in our analysis. Particularly when bearing in mind the distinction between statistical and clinical significance as detailed above, this large literature supports our current results and dosing suggestions when measuring FFR.

Finally, investigating the effect of saline and contrast medium, we found that intracoronary injections of contrast medium and saline increased Doppler flow velocity, with contrast's being more potent. Extensive prior work has demonstrated the hyperemic effect of contrast medium, but mainly used older agents different from modern, low osmolality formulations. Recent data suggest that current contrast agents produce meaningful (submaximal-) hyperemia for measurement, allowing close estimation of the value of FFR (CONTRAST trial, clinicaltrials.gov NCT02184117). [124] We note only that our results imply that both saline and contrast produce some degree of hyperemia, presumably partially via transient hypoxia from replacement of oxygenated blood and partially by stimulating endothelial paracrine pathways.

Even though we believe that these data strongly confirm the optimal dosing of intracoronary adenosine to achieve maximal hyperemia, some limitations have to be acknowledged. We did not measure FFR simultaneously due to less robust technology for continuous and combined pressure/flow measurements, but instead used standard physiology to relate changes in flow to changes in pressure loss. A number of additional limitations have to be taken into account. While our sample size was modest, it was of

comparable magnitude to prior dose-response work using intracoronary adenosine and Doppler sensors. Although each patient served as his or her own control to generate a dose-response curve for intracoronary adenosine, we did not measure the Doppler flow velocity response to intravenous adenosine or intracoronary papaverine. Yet, several other studies have shown that intravenous adenosine and intracoronary adenosine provided similar degrees of hyperemia. [95;110] Additionally, we did not explore intracoronary adenosine doses above 500 μg, although our results suggest diminishing returns from such ultra-high levels. Also, the scientific rigor of the study would have been increased by a randomization of the various dosages of adenosine. Finally, only 'normal' arteries were studied. Yet, the complete dose-response effect on flow can be investigated only in vessels with minimal or no epicardial resistance. In 'critical' stenoses, when the microvascular resistance reserve is already exhausted at rest to compensate for the high epicardial resistance, the flow cannot increase further. Therefore an FFR model is sub-optimal to investigate the full range of effects of adenosine.

Summarizing, based on the findings of our dose-response analysis a clear recommendation can be made for the optimal dosing of adenosine for the measurement of FFR: 100 μg in the right coronary artery and 200 μg in the left coronary artery provides with reliable maximal hyperemia. These dosages do not induce any significant side-effects, achieve >95% of maximum hyperemia and are clinically indistinguishable from higher dosages when applied for FFR measurements. While lower doses are less reliable to reach maximal hyperemia, therefore inducing inaccuracy of our measurement with potential underestimation of lesion severity, administration of higher doses is unnecessary and discouraged, because of irrelevant changes in the level of hyperemia but higher rate of potential side-effects.

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8. Conclusion

This work investigated important topics regarding FFR measurement, and we believe, a couple of crucial questions have been answered, potentially facilitating an even broader acceptance of the technology.

With extensive dose-response analysis we managed to give clear practical recommendation for measuring FFR, by defining the optimal dosages for intracoronary adenosine to reliably achieve maximal hyperemia. Data confirmed that any decrease in dosages or any attempt with semi- or non-hyperemic measurements impacts negatively the accuracy, while further increase in adenosine dosages does not have any benefit in terms of accuracy, therefor can be considered as non-sense.

Our data gives the clear answer and closes the debate about applicability of FFR in patients with severe heart failure. Data confirm, even though the formula of FFR calculation is simplified in terms of neglecting the right atrial pressure for the sake of easier applicability, but this has no relevant impact on the FFR value, not even in patients with pathologically elevated central venous pressures.

We confirmed on the largest population so far what massive discrepancies can be observed between angiographic severity of a stenosis and its true ischemic potential. This finding provides with the conceptual background for the strong clinical outcome data, supporting FFR-guided revascularization strategies above angio-based decision-making, and therefore strongly discouraging any purely anatomy guided revascularization attempts.

We believe these findings have the potential to have relevant impact on our future clinical practices.

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9. Summary

Fundamental studies have shown that benefit from revascularization can be only expected when it eliminates ischemia. Accordingly, revascularization guidelines recommend that indication or deferral of revascularization have to be based on functional assessment by fractional flow reserve (FFR) during coronary angiography.

FFR is defined as the ratio of hyperemic myocardial blood flow in the presence of a stenosis to the same but in the absence of any stenosis. It is calculated by the ration of distal coronary mean pressure to aortic mean pressure during maximal hyperemia.

Despite powerful outcome data and the highest level of recommendation the adaptation by the interventional community is still limited. This work answered potential questions, which might be the background of hesitant application of the technology.

First, we confirmed on the largest population so far what massive discrepancies can be observed between angiographic stenosis severity and its true ischemic potential. Misinterpretation of lesion severity, which occurs in one third of all angiogram-based decisions, might lead to over- or under-treatment of the patients, both resulting in excess of hazard. This finding provides with the conceptual background for the strong clinical outcome data, supporting FFR-guided revascularization strategies and strongly discouraging any purely anatomy guided revascularization attempts.

Second, our data closes the debate about applicability of FFR in patients with severe heart failure. We confirmed, even though the formula of FFR calculation is simplified in terms of neglecting the right atrial pressure, but this has no relevant impact on the FFR value, not even in patients with pathologically elevated central venous pressures.

Finally, with extensive dose-response analysis we managed to give clear practical recommendation, by defining the optimal dosages for intracoronary adenosine. We found that 100 μg in the right coronary or 200 μg in the left coronary can reliably induce maximal hyperemia. We confirmed that any decrease in dosages or any attempt with semi- or non-hyperemic measurements impacts negatively the accuracy, while further increase in adenosine dosages does not have any benefit in terms of accuracy, therefore both can be considered as non-sense.

We believe these findings have the potential to have relevant impact on our future clinical practices by clarifying fundamental questions about application of FFR.

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10. Összefoglalás

Vizsgálatok igazolták, hogy a revaszkularizációtól csak akkor várható előny, amennyiben azzal igazoltan iszkémiás kockázatot szűntetünk meg. Ennek megfelelően az ajánlások egyértelműen javasolják, hogy az egyes szűkületekről hozott terápiás döntést funkcionális megítélésre kell alapozni, melynek 'gold standard' invazív módszere a frakcionális áramlási rezerv (FFR).

Bár a módszert jelentős klinikai eredmények támasztják alá és ennek megfelelően a legmagasabb szintű ajánlásokban szerepel, mégis az intervenciós társadalomban az elfogadottsága és az alkalmazása ehhez képest korlátozott. A dolgozatban olyan kérdéseket és problémákat válaszoltunk meg, melyek esetlegesen a technológiával szembeni bizalmatlanság hátterében állhattak.

Először, az irodalomban eddigi legnagyobb betegcsoporton igazoltuk, hogy az angiográfiás mérések jelentős devianciát mutatnak a szűkületek valós funkcionális jelentőségéhez képest. Így az szűkületek hibás megítélése, ami az angiográfiára alapozott döntések egy harmadában fordul elő, túl- vagy alul kezeléshez vezetnek, és ezáltal kockázatnak teszik ki a betegeket. Ez az alapvető eredmény alátámasztja a nagy klinikai vizsgálatokat, melyek igazolták az FFR vezérelt revaszkularizációs stratégiák klinikai előnyét és rámutattak az pusztán angiográfiára alapozott kezelés kockázataira.

Másodszor, a dolgozat anyaga pontot tesz egy vita végére, mely megkérdőjelezi az FFR alkalmazhatóságát szívelégtelen betegek esetében. Bár az FFR kalkulációjakor egyszerűsítés céljából a jobb pitvari nyomásértéktől hagyományosan eltekintünk, kimutattuk ennek az értéknek a beszámítása még jelentősen emelkedett jobb pitvari nyomásértékek esetén sem változtatja releváns mértékben az FFR értékét.

Végezetül, átfogó dózis-hatás vizsgálatunk alapján egyértelmű ajánlást tesz a dolgozat az intrakoronáriás adenozin optimális dózisára. Azt találtuk, hogy 100 μg jobb koszorúérbe- vagy 200 μg bal koszorúérbe adott adenozin megbízható és reprodukálható maximális hyperémiát indukál. Igazoltuk, hogy a dózis csökkentése, illetve non- vagy semi-hyperémiás mérések a diagnosztikus pontosság csökkenéséhez vezetnek. Ezzel szemben a dózis további emelése nem jár járulékos diagnosztikus előnnyel. Ezért mindkét módszer indokolatlan és értelmetlen.

Mindezek alapján a dolgozat alapvető kérdéseket válaszol meg az FFR alkalmazásával kapcsolatban, így eredményei jelentős mértékben befolyásolhatják a klinikai gyakorlatot.

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12. Bibliography of the candidate's publications

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