COronary and MICrocirculatory measurements in patients with Aortic valve Stenosis, the COMIC AS study.

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LIST OF ABBREVIATIONS

AE	Adverse Event
AS	Aortic Stenosis
CAD	Coronary Artery Disease
CFR	Coronary Flow Reserve
CRF	Case Report Form
CV	Cardiovascular
FFR	Fractional Flow Reserve
GLS	Global Longitudinal Strain
IC	Informed consent
iFR	Instantaneous wave-free ratio
IMR	Index of Microcirculatory Resistance
IRB	Institutional Review Board
LVEDP	Left Ventricular End Diastolic Pressure
LV	Left Ventricular
MACE	Major Adverse Cardiovascular Events
MI	Myocardial Infarction
MVD	Microvascular Disease
NA	Not Applicable
NHPR	Non-Hyperemic Pressure Ratio
NSTEMI	Non-ST-elevation Myocardial Infarction
RFR	Resting full-cycle ratio
SAE	Serious Adverse Event
SAP	Statistical and Analytical Plan
SPECT	Single-photon emission computed tomography
STEMI	ST-elevation Myocardial Infarction
SAVR	Surgical aortic valve replacement

TAVI/R Transcatheter aortic valve implantation/ Replacement

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with Good Clinical Practice (GCP, ICH E6). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants.

Principal Investigator: Prof. Dr. Keir McCutcheon

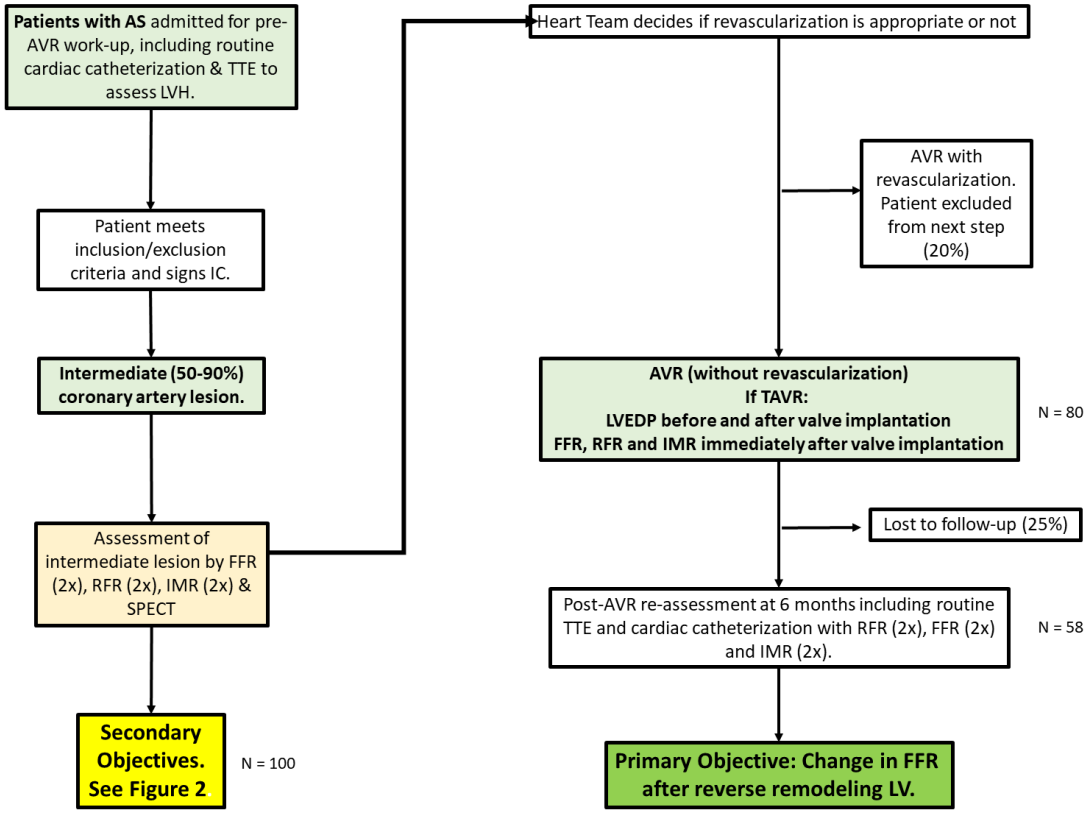
Signed:  Date: 15-04-2021

PROTOCOL SUMMARY

Aortic stenosis (AS) is the most common primary valve disease leading to surgery or catheter intervention in Europe, with a growing prevalence due to the aging population. Severe AS is associated with significant morbidity and mortality and is treated by surgical valve replacement (SAVR) or transcatheter aortic valve implantation (TAVI). However, significant coronary artery disease (CAD) is reported in more than 60% of patients with AS, and this concomitant CAD needs to be correctly assessed because untreated significant CAD is associated with adverse outcomes. Current guidelines recommend intra-coronary pressure measurement using either fractional flow reserve (FFR) or non-hyperemic pressure ratios (NHPR) for the assessment of intermediate coronary artery lesions before revascularization. However, correctly identifying hemodynamically significant coronary stenoses by FFR or NHPR is difficult in patients with AS because of the hypertrophic remodeling related to the valve pathology, which interferes with coronary blood flow in the microcirculation and reduces the accuracy of FFR and NHPR. This has resulted in substantial controversy in this field of coronary artery disease and there is currently no clear guideline for the management of AS patients with concomitant CAD. Recent technological advances now permit the concomitant evaluation of FFR/NHPR and microcirculatory function at the time of cardiac catheterization. Using this technology, we will simultaneously evaluate the hemodynamic significance of coronary stenoses (using FFR and a NHPR - Resting Full cycle Ratio [RFR]) and the microcirculatory function (Index of Microcirculatory Resistance, IMR) in patients with AS and CAD prior to SAVR/TAVI. One hundred patients with AS and intermediate CAD will be included in the study. Of them Eighty will undergo AVR and at least 58 will undergo the follow-up at 6 Months. During routine pre-valve replacement cardiac catheterization, measurement of intermediate CAD lesions (by FFR and RFR) and IMR will be performed in duplicate using new software. These measures of CAD severity will be compared with a gold standard non-invasive test (single photon emission computed tomography [SPECT]). This will validate the use of RFR and determine if IMR can be used to improve the ischemia prediction of FFR. In addition, the patients undergoing TAVI the LVEDP will be measured immediately before and after valve implantation and FFR, RFR and IMR will be measured after valve implantation. Subsequently, when these patients undergo a post-intervention (whether SAVR or TAVI) evaluation 6 months after valve replacement, the effect of valve replacement on FFR, RFR and IMR (again in duplicate) will be determined. This will be the first study measuring these parameters before and 6 months after valve replacement. The data will be used to calculate a 'corrected' FFR (or RFR) based on microcirculatory function in these patients, which can then be used in the future to correctly evaluate CAD pre-intervention in patients with AS. This will translate into more efficient management of patients with AS. Fewer of these patients will unnecessarily undergo coronary artery bypass grafting or stenting when the CAD does not need to be treated and, conversely, in those who do require treatment of the CAD, revascularization can be carried out during a single surgery/intervention, avoiding multiple high risk procedures in patients who are already at high peri-operative/peri-interventional risk.

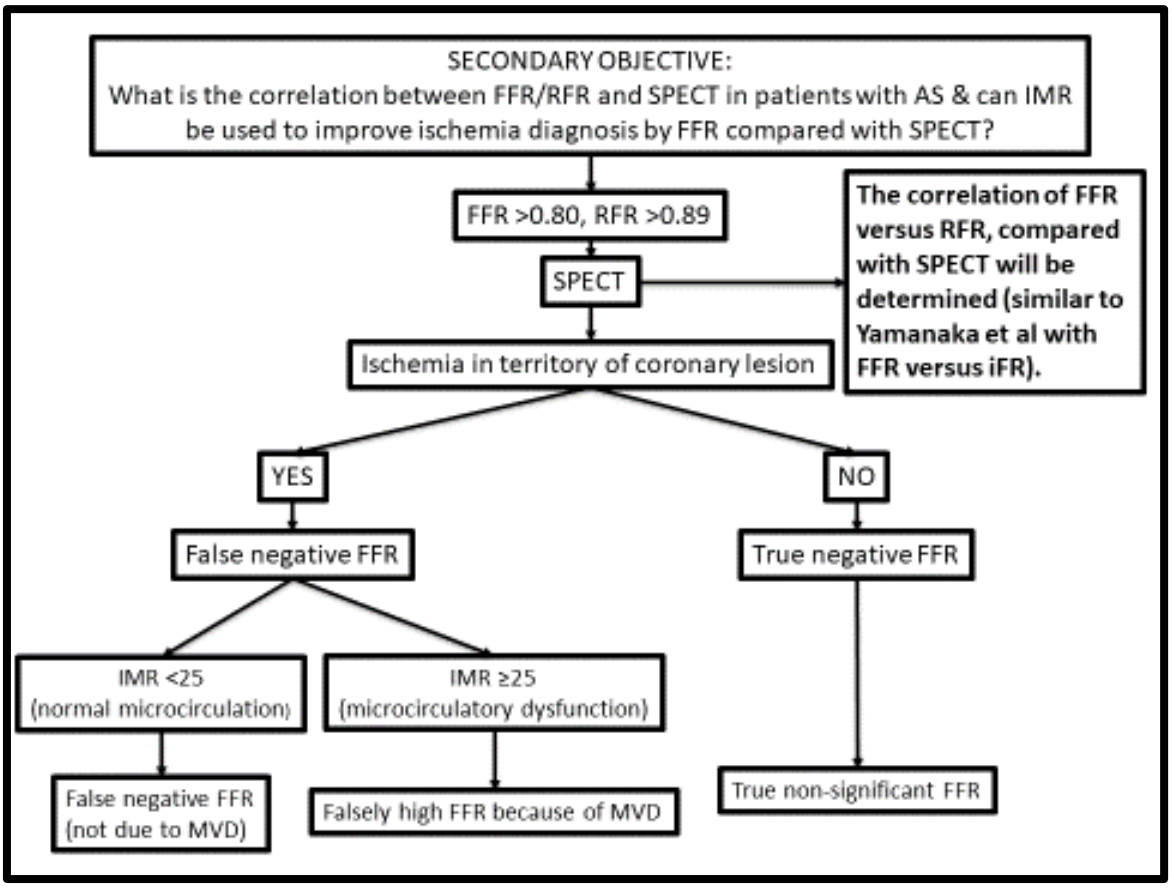
SCHEMATIC OF STUDY DESIGN

FIGURE 1: Study flow.



AS, aortic stenosis; AVR, aortic valve replacement (by surgery or transcatheter aortic valve implantation); TAVR, Transcutaneous aortic valve replacement; TTE, trans-thoracic echocardiography; IC, informed consent; SPECT, Single-photon emission computed tomography; FFR, fractional flow reserve; RFR, resting full cycle ratio; IMR, index of microcirculatory resistance; LV, left ventricle.

FIGURE 2: Secondary Objective.



FFR, fractional flow reserve; RFR, resting full cycle ratio; IMR, index of microcirculatory resistance; iFR, instantaneous wave-free ratio; SPECT, Single-photon emission computed tomography; AS, aortic stenosis; MVD, microvascular/microcirculatory dysfunction. Yamanaka *et al*.[1] compared FFR versus iFR with SPECT as the standard for ischemia.

SYSTEMATIC PROTOCOL STUDY SYNOPSIS

Title	COronary and MICrocirculatory measurements in patients with Aortic valve Stenosis, the COMIC AS study.
Design	A single centre, prospective, cohort study assessing the changes in intra-coronary flow and pressure before and 6 months after aortic valve intervention.
Objectives	**Primary endpoint:** 1. Aortic valve intervention results in a significant change in fractional flow reserve (FFR) 6 months after SAVR/TAVI. **Secondary endpoints:** 2. Measurement of IMR in patients with AS prior to intervention and 6 months after TAVI/SAVR. 3. Corrected FFR (based on baseline IMR) correlates with the post-intervention FFR. 4. IMR at baseline improves the ischemia prediction of FFR (compared with SPECT). 5. Comparison of the diagnostic performance of FFR and RFR with SPECT ischemia.
Treatment	There is no study-related intervention. Aortic valve intervention is carried out according to normal clinical practice.
Follow-up	Patients will under-go clinical assessment, TTE and cardiac catheterization 6 months after aortic valve intervention.
Inclusion criteria	1. Patient undergoing the procedure is older than 18 years, has severe aortic valve stenosis (according to ESC guidelines) and is planned for cardiac catheterization as part of the pre-operative (SAVR) or pre-percutaneous (TAVI) work up.

	2. The patient has an intermediate (50-90%) coronary lesion that requires further evaluation. 3. The patient undergoing the procedure is male, or if female, has no childbearing potential or is not pregnant.
Exclusion criteria	1. The procedure is an emergency and/or the patient is unstable. 2. Pregnancy or lactation 3. Haemodynamically unstable patients 4. Killip class III-IV heart failure 5. Previous coronary artery by-pass in the artery being assessed 6. Contra-indications for adenosine administration: severe asthma or pre-existing type 2 AV-block 7. No significant coronary artery disease (<50% stenosis on angiography). 8. Critical coronary artery disease deemed by the Heart Team to require immediate revascularization 9. Patients will be excluded from the SPECT study (secondary objective) if they have a left main coronary stenosis >50%, triple vessel disease, previous myocardial infarction in the same coronary artery & tandem lesions (separated by >10mm) requiring independent evaluation in the same coronary artery since these factors interfere with the SPECT analysis.
Number of patients	• 80 patients with pre- and post-intervention (TAVI or SAVR) catheterizations o Fifty Eight t (58) patients with pre- and post-interventions catheterizations will be sufficient to detect a reduction in the FFR from 0.82 to 0.79 with an alpha level of 0.05 and power of 0.80. A total sample of 100 with baseline measurements and subsequent 80 patients who will undergo (T)AVR without revascularisation and are eligible for a pre-and post-

	intervention catheterization will allow for a 25% drop-out during the follow up phase of the study. o This flow with patient numbers and dropout is illustrated in figure 1 for clarity.
Study duration	3 years (or earlier when the 80 pt's with an aortic valve procedure without revascularisation will be included)

1. BACKGROUND

Aortic stenosis (AS) is the most common primary valve disease leading to surgery or catheter intervention in Europe, with a growing prevalence due to the aging population. Severe aortic valve stenosis, defined by a mean gradient over the aortic valve of greater than 40mmHg or an aortic valve area of less than 1.0cm^2,[2] is associated with significant morbidity and mortality and is treated by surgical valve replacement (SAVR) or transcatheter aortic valve implantation (TAVI). However, significant coronary artery disease (CAD) is reported in more than 60% of patients with AS,[3] and needs to be correctly assessed because untreated significant CAD is associated with adverse outcomes.[4,5]

Current guidelines recommend intra-coronary pressure measurement using either fractional flow reserve (FFR) or non-hyperemic pressure ratios (NHPRs) for the assessment of intermediate coronary artery lesions before revascularization.[6] A FFR of ≤0.80 or a NHPR of ≤0.89 is considered to indicate that a coronary lesion is significant. Non-hyperemic pressure ratios include the instantaneous wave-free ratio (iFR) and the resting full cycle ratio (RFR), which have been shown to be comparable.[7] However, correctly identifying hemodynamically significant coronary stenoses is difficult in patients with AS because of the hypertrophic remodelling related to the valve pathology.[8] Aortic stenosis is associated with a significant increase in left ventricular (LV) mass resulting in pathological changes in coronary and LV hemodynamics, which are associated with coronary microvascular dysfunction.[9,10]

With the development of microvascular dysfunction, intra-coronary pressure measurements become less reliable and, currently, whether FFR, or the NHPRs, can reliably detect myocardial ischemia in patients with severe AS is controversial, making it difficult for physicians to correctly determine whether or not coronary lesions should be revascularized prior to TAVI or at the time of SAVR. Yamanaka *et al*.[1] recently reported a strong correlation of iFR with FFR in patients with

AS and intermediate CAD. They determined that the optimal iFR threshold for detecting ischemia compared with 99mTc single-photon emission computed tomographic (SPECT) myocardial perfusion imaging was 0.82, while the cut-off for FFR was 0.83. This is in contrast to the large, outcome-based randomized controlled studies in patients without AS,[11,12] and different to the current guideline cut-offs of ≤0.89 for resting indices and ≤0.80 for FFR.[6] The RFR threshold for detecting ischemia has not yet been determined in patients with AS.

A more comprehensive study with performance of intra-coronary measurements before and after TAVI may help determine the effect of adverse LV remodelling and microvascular dysfunction on the hemodynamic measurement of coronary artery lesions in patients with severe AS. However, the timing of the follow-up measurements has not been clearly defined. Several studies have performed acute measurements pre- and post-TAVI[8,13-15] but no invasive study (measuring FFR and/or resting indices) has been performed after allowing adequate time for reverse remodelling in order to really determine the effect of microvascular reverse remodelling on intra-coronary hemodynamic measurements. Exactly how long is needed after TAVI to ensure adequate myocardial and microvascular remodelling has occurred in order to appropriately use intra-coronary hemodynamic measurements is not known at this stage. One MRI-based study demonstrated a significant reduction in indexed LV mass from 76g/m² to 68g/m² within a few days.[16] In another study, echocardiographic ejection fraction and LV global longitudinal strain improved significantly when measured 10 days after TAVI.[17] However, indexed LV mass improvement was only significant during long-term (months-to-years) follow up. In a more recent, echocardiographic-based study, indexed LV mass decreased from 117 g/m² to 100 g/m² in a surgical aortic valve replacement (SAVR) group and 114 g/m² to 108 g/m² in a TAVI group after 3 months with further reductions at one year.[18] Whether these global changes in LV remodelling translate into improvements in microvascular function, and hence improve the diagnostic accuracy of FFR is not yet known.

If TAVI/SAVR results in a significant improvement in LV remodelling and microvascular function, it should be expected that the current invasive gold-standard for measuring microvascular resistance (IMR; index of microcirculatory resistance)[19] would improve several months after the valve intervention. If this is the case then the FFR measurement should be more accurate (as it is in patients without aortic stenosis). The question is whether a pre-TAVI/SAVR index (based on a combination of FFR and IMR) could be used in the future for assessing intermediate lesions in these patients. Developing a "corrected" FFR measurement of intermediate coronary artery lesions based on the presence or absence of microvascular dysfunction would be very useful in the pre-operative work-up of patients with aortic stenosis and would have important prognostic implications. This will be accomplished by first determining the FFR 6 months after intervention (TAVI or SAVR), when it is anticipated that the microvascular function would have returned to normal,[20] and comparing this with the FFR prior to the intervention to determine if there is a significant change in the FFR after intervention. At the same time microvascular function will be assessed in order to develop a "corrected" FFR, which can be used in the future to determine whether or not coronary lesions need to be revascularized in patients with aortic stenosis.

The theoretical model of a serial stenosis by the AS and the coronary narrowing in our study is explained in the figure 1 in the publication of Zelis at al.[24] It is expected that the FFR will indeed decrease after TAVI and that there will be an acute (change of intercept) and chronic (change of slope) effect of the procedure on the coronary resistance. To separate these two issues we will try to measure the FFR immediately after TAVI and 6 months after TAVI. In addition the left ventricular filling pressure/ end-diastolic pressure (LVEDP) will be measured during the TAVI procedure immediately before and after valve implantation. The measurement (the delta LVEDP) will be an important marker of the "intercept" that changes acutely. To make sure the measurements of IMR, FFR and RFR are correct these will be performed twice and an average value will be used. This will also allow us to test the reproducibility.

1. STUDY OBJECTIVES

Primary outcome

1. Null hypothesis: TAVI/SAVR does not result in a significant change in FFR/RFR 6 months after intervention (Figure 1).
    a. If the null hypothesis is upheld, this suggests that FFR/RFR measurements in patients with AS is valid even in the presence of severe AS.
    b. If there is a significant decrease in FFR/RFR 6 months after aortic valve treatment, this suggests that the use of FFR/RFR to evaluate intermediate lesions in patients with severe AS is not valid and requires further investigation.

Secondary outcomes

2. Measurement of IMR in patients with AS prior to intervention and 6 months after SAVR/TAVI.
    a. Description of the range of IMR values in a cohort of patients with severe AS.
        i. Do all patients with severe AS have high microvascular resistance as assessed by IMR?
        ii. Does IMR change significantly 6 months after TAVI/SAVR? If there is a significant decrease in IMR then this indicates that microcirculatory function improves 6 months after aortic valve intervention.
3. Null hypothesis: the “corrected” FFR (at baseline; based on baseline IMR) does not correlate with the post-intervention FFR.
    a. If the null hypothesis is upheld then a correction of FFR using baseline IMR does not improve the validity of FFR in patients with severe AS.
4. Null hypothesis: using IMR at baseline does not improve the ischemia prediction of FFR. Ischemia is measured by SPECT (Figure 2).
    a. If the null hypothesis is upheld then IMR does not improve stratification of coronary ischemia based on FFR.

b. If a significant proportion of patients with SPECT ischemia have a raised IMR this would support the hypothesis that IMR can be used to correct falsely high (negative) FFR measurements.

5. Comparison of the diagnostic performance of FFR and RFR with SPECT ischemia (and compare with findings of Yamanaka *et al*[1]).
6. Reproducibility of the FFR, RFR and IMR

Expected outcomes

Primary outcome: If the null hypothesis is upheld, this suggests that FFR/RFR measurements are valid even in the presence of severe AS.

Secondary outcomes

1. A description of the range of IMR values in a cohort of patients with severe AS will be a novel and important contribution to the field of interventional cardiology. Furthermore, the change in IMR (microvascular function) in response to valve replacement will be an important finding in this study. If there is a significant decrease in IMR then this indicates that microcirculatory function improves 6 months after aortic valve intervention. Since microvascular function predicts outcomes in patients with AS, IMR evaluation at 6 months after TAVI/SAVR might provide an additional method to risk stratify these patients even after correction of the valve pathology.[21]
2. If the "corrected" FFR (at baseline; based on baseline IMR) does not correlate with the post-intervention FFR then a correction of FFR using baseline IMR does not improve the accuracy of FFR in patients with severe AS.
3. Compared with SPECT, a correlation of FFR and RFR will be an important contribution. Firstly, this will be a validation of RFR as a resting measure of ischemia in AS patients. Secondly, these data will support the data of a previous study[1] with a different resting measure of ischemia (iFR) or the findings may be in conflict with the previous study, which would be important.

4. Using SPECT as a standard of myocardial ischemia, IMR may improve the ischemia prediction of FFR. This would be an extremely important finding, supporting the routine use of IMR in patients with microvascular disease when coronary artery lesions need assessment by FFR. Because AS is an important clinical condition with reversible microvascular dysfunction, this provides an excellent naturally occurring model for the study of microvascular function and CAD. These findings will be useful in other groups of patients who also have microvascular dysfunction (including patients with heart failure, obesity, diabetes, liver disease, chronic kidney disease and heart transplant patients). Being able to factor-in microvascular dysfunction (using simultaneously measured microvascular resistance) at the time of FFR measurement will permit accurate assessment of the severity of CAD in these patients. This study has the potential to revolutionize the assessment of coronary artery lesions, not just in patients with AS, but in all patients with CAD since the validity of CAD assessment by FFR/NHPRs may, indeed, depend on microvascular function.
5. We hypothesize that in AS patients the measurements will be reproducible such as in other patients, however we expect the RFR to be more reproducible than the FFR which we expect to be more reproducible than the IMR.

3. STUDY DESIGN & INTERVENTIONS

3.1 Study Design

A single centre, prospective, cohort study.

3.2 Study duration

The anticipated duration of the study will be 3 years.

3.3 Patient population

Patients undergoing routine cardiac catheterization during preparation for TAVI or SAVR, who are found to have coronary artery disease, will be approached to take part. A transthoracic echocardiography (TTE) will take place at baseline and at 6 months. A TTE is part of the routine care and almost without risk. The TTE will assess systolic function, diastolic function, right and left ventricular function, valvulair function, fluid status, pulmonary pressures and shear wave analysis to assess the stiffness of the heart . These two extra TTE's will be necessary to standardize the assessment of the valve pathology, to ensure our patients are good candidates and to assess the status post intervention in comparison to before the valve procedure Cardiac catheterization is routinely performed in these patients and intra-coronary hemodynamic measurements are routinely used during the assessment of coronary artery disease. SPECT is used to confirm the presence of ischemia. Baseline measurements of coronary artery disease and coronary microvascular function (IMR) will be carried out during the baseline cardiac catheterization if intermediate coronary artery lesions (defined as a coronary artery stenosis in the proximal part of a major coronary artery of 50-90% stenosis). During the six-month cardiac catheterization the coronary artery disease is assessed. During this procedure an additional measure of microvascular function (IMR) will be performed. FFR is routine clinical practice, but the additional measurement of IMR will be investigatory.

3.4 Informed consent process

Informed consent should be obtained in accordance to the applicable regulations, the declaration of Helsinki and Good Clinical Practice. All potential patients must give their written consent prior to any study specific procedures. If a patient might be eligible for the study (after a first check of the inclusion/exclusion criteria), the investigator or delegated staff should approach the patient to obtain the written informed consent. During this interview the background of the proposed study and the benefits and risks of the study participation should be explained in detail to the patient. It has to be emphasized that a patient's participation in the trial is voluntary and that the patient may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the patient is otherwise entitled. The patient must be given ample time to read the whole patient information and to address questions before signing the consent form. The patient information and a copy of the signed consent form will be handed out to the patient. The patient information as well as the patient informed consent will have the approval of the institution's Review Board and/or Ethics Committee (IRB/IEC) prior to their usage.

3.5 Inclusion criteria

1. Patient is older than 18 years and is planned for a cardiac catheterization as part of the work up for TAVI or SAVR.
2. Patient has severe aortic valve stenosis as defined by the European Society of Cardiology.[2]
3. The coronary angiogram cardiac catheterization is elective.
4. The patient has an intermediate (50-90%) coronary lesion that requires further evaluation.
5. The subject is male, or if female, has no childbearing potential or is not pregnant.

3.6 Exclusion criteria

1. The procedure is an emergency and/or the patient is unstable.
2. Pregnancy or lactation
3. Haemodynamically unstable patients

4. Killip class III-IV
5. Previous CABG
6. Contra-indications for adenosine administration: severe asthma or pre-existing type 2 AV-block
7. No significant coronary artery disease (<50% stenosis on angiography).
8. Critical coronary artery disease deemed by the Heart Team to require revascularization.
9. Patients will be excluded from the SPECT study (secondary objective) if they have a left main coronary stenosis >50%, severe triple vessel disease, previous myocardial infarction in the same coronary artery & tandem lesions (separated by >10mm) requiring independent evaluation in the same coronary artery since these factors interfere with the SPECT analysis.

3.7 Screening and enrolment process

Only if all inclusion criteria are met/exclusion criteria are not met, can the patient be included in the study.

3.8 Methodology

Figure 1 summarizes the study flow. Patients admitted electively for a valve work-up and patients admitted through the Emergency department that have to undergo a valve work-up will be approached to take part in the study. All these patients undergo routine cardiac catheterization during preparation for TAVI or SAVR. If they are found to have intermediate coronary artery disease on the angiography, measurments to assess the severity of the lesions and microvascular function (further explained beneath) will be performed during the catheterisation. . Cardiac catheterization is routinely performed in these patients and intra-coronary hemodynamic measurements are routinely used during the assessment of coronary artery disease. SPECT is also used to confirm the presence of ischemia. Baseline intra-coronary measurements of coronary artery disease and coronary microvascular function (IMR) will be carried out in duplicate during the baseline cardiac catheterization if intermediate coronary artery lesions (defined as a coronary artery stenosis in the proximal part of a major coronary artery of 50-90% stenosis by quantitative coronary analysis).

During routine cardiac catheterization, patients with intermediate coronary artery lesions, will undergo hemodynamic assessment of the coronary stenosis according to current ESC guidelines.[6] This entails the use of a pressure wire (Pressure Wire X, Abbott Medical) in the coronary artery distal to the stenosis in order to determine the pressure gradient over the stenosis. Prior to induction of hyperemia, Resting Full cycle Ratio (RFR) is measured before determining fractional flow reserve (FFR). In order to measure a FFR, adenosine at 140µg/kg/min is administered via a peripheral venous infusion catheter. This is routine clinical practice. To address for factors that might cause small changes in these measurements, all measurements will be done twice, without an extra risk to the patient. Two measurements are frequently performed in clinical practice to get a robust measurement. This will also allow us to test the reproducibility of our measurements.

At this time three boluses of room-temperature saline are administered in order to measure the intra-coronary flow during hyperemia by the method of thermodilution.[19] This extra step, which is investigational, adds no additional risk or cost to the patient. However, it permits the calculation of flow and microvascular resistance or IMR (index of microvascular resistance) using Coroflow® software (Coroventis, Uppsala, Sweden). IMR is already clinically indicated in several other groups of patients (with suspected microvascular dysfunction) and is used on a routine basis in patients in our cardiac catheterization laboratory.

In order to assist the Heart Team in deliberation over the ischemic importance of intermediate coronary lesions, SPECT imaging is clinically indicated during the pre-operative/interventional work up. This will be carried out in the Department of Nuclear Medicine according to routine clinical practice under the guidance of Dr. Sander Jentjens. If adenosine cannot be used then regadenoson will be used.[22]

Intra-coronary assessment of intermediate lesions is part of routine clinical practice. The decision to revascularize and the method of revascularization (percutaneous coronary intervention versus coronary artery bypass grafting) of patients with aortic stenosis based on intra-coronary measurements and non-invasive measures of ischemia (SPECT) is discussed by the Heart Team (including the cardiologist and cardiothoracic surgeon) and forms part of routine clinical practice. In patients who do not undergo immediate revascularization, the residual CAD should be reassessed after SAVR/TAVI. Current controversy about the validity of both intra-coronary and non-invasive tests in patients with AS necessitates further evaluation (after valve intervention) since coronary lesions should not be unnecessarily treated (Class III recommendation in the ESC guidelines[6]).

In the patients who will undergo TAVI measurements of LVEDP will be carried out during the TAVI procedure immediately before and after valve implantation. As the catheters are already present in the ventricle of the patient at this point this does not require and extra intervention

of risk (the pressure measured by the catheter will simply be recorded for the study data). Immediately following TAVI, intracoronary hemodynamic assessment of the intermediate study lesions will be performed with measurement of FFR, RFR and IMR. For this assessment the same arterial access used during TAVI will be used. Finally, during the six-month cardiac catheterization the coronary artery disease is assessed. During this procedure additional measurements of microvascular function (IMR) will be performed. Again, FFR is routine clinical practice, given the ambiguity of earlier measurements in the presence of aortic valve stenosis, but the additional measurements of IMR will be investigatory. As discussed before the measurements will be performed in duplicate.

Laboratory Procedures/Evaluations

- At the time of admision routine laboratory blood and urine tests are performed including complete blood count, WBC differentiation, electrolytes, creatinine, , INR, pro-BNP, troponine, HbA1c, thyroid function and etc. This data will be collected.
- Biological samples will be collected with everybody that signs the informed consent, at baseline and at 6 months follow-up.
- A peripheral blood sample (4.0mL EDTA, 2.7 mLcitrate; 4.5mL LithiumHeparine, 5.0 mL Serum tube 2.5mL PaxGene® RNA tube) will be obtained to assess for biomarkers and platelet function. A urinary sample (14 mL) will be collected to asses for biomarkers related to aortic valve stenosis or microvascular dysfunction through the use of proteomic analysis.
- Approval from the biobank has been obtained with a biobank application form (BB-GEN002-F003) filled in and signed by the PI and Biobank Manager.

4. SCHEDULE OF EVENTS TABLE

Procedures	Screening	Baseline	At the time of TAVI	6-month follow-up
Assess inclusion and exclusion criteria	x			
Informed consent	x			
Enrolment	x			
Patient characteristics		x		x
Transthoracic echocardiography		x		x
Cardiac catheterization and FFRs		x		x
IMR measurements during Catheterization		x		x
Blood sampling		x		x
Urine sampling		x		x
SPECT		x		(x)
Transthoracic echocardiography		x		x
LVEDP measurement (2x)			x	
IMR and FFR measurements			x	
Adverse events		x		x
Serious adverse events		x		x

Timeline

Objective	First half 2020	Second half 2020	2021	2022	First half 2023	Second half 2023

Ethics approval						
Patient recruitment						
Second catheterization						
Analysis Secondary Objective (FFR versus RFR analysis)						
Analysis Primary Objective (FFR changes at 6 months)						

5. FUNDING

Item	Cost per item (€)	Number needed	Total cost (€)
PaxGene tubes	11	400	4400,00
Regadenasone	140	80	11 200,00
Pressure Wire X	220	40	8 800,00
Total cost of study			**24 400,00**

6. ADVERSE EVENTS

During the course of this clinical investigation adverse events might occur. These adverse events will be determined by the investigator during the scheduled patient follow-ups. All serious adverse events must be reported within 24 hrs from acknowledgement. Other adverse events should only be reported whenever they affect one or more of the predefined endpoints of the study. These include all myocardial infarctions (any definition), stent thromboses and coronary revascularizations, even when these would not result in death, hospitalization, prolonged hospitalization or persistent disability. These adverse events must be reported on the respective Adverse Event Form.

6.1 Adverse events definition and classification

An adverse event (AE) is any untoward medical occurrence in a patient whether or not related to the investigated treatment. Any current condition that is recorded as a pre-existing condition either in the medical history of physical examination section, unless there is a change in nature, severity, or degree of incidence, is not an AE.

Classification of the adverse events has to be done according to the following criteria:

Intensity

Mild: patient's daily activities are not limited

Moderate: patient's daily activities are limited

Severe: patient is not able to perform daily activities

Relation to Study Device

1=not related

2=possibly related

3=probably related

4=definitely related

Relation to the study devices will be assessed by help of the following criteria: timely relation of the event to the procedure, known risks of the device and intervention, concomitant diseases, concomitant measures and medication, other possible explanations, as applicable. Additional criteria might exist depending on the study setup and adverse event. Documentation about judgement and causality assessment has to be available for review in the source data.

Relation to procedure

1=not related

2=possibly related

3=probably related

4=definitely related

Relation to the procedure will be assessed by help of the following criteria: timely relation of the event to the procedure, known risk of the intervention, concomitant diseases, concomitant measures and medication, other possible explanations, as applicable. Additional criteria might exist depending on the study setup and adverse event. Documentation about judgement and causality assessment has to be available for review in the source data.

Seriousness of the Adverse Event

In case any of the following criteria is applicable to the Adverse Event it classifies as SAE. The Adverse Event:

- results in death;
- is life threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect

This also applies to adverse events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above.

The assessment of “seriousness” is independent from any relation to the investigational device.

Hospitalization is defined by a medical need for inpatient hospitalization. For procedures that are commonly performed ambulant and require inpatient hospitalization solely based on nonmedical needs (e.g. logistical reasons) this criterion could be considered not applicable by the investigator. Proper documentation of the reasons for decision is required in this case.
Events that are planned (e.g. elective surgery) and documented prior to study entry will not be recorded as (serious) adverse event. Medication received during this activity will be recorded.

6.2 Adverse device effects/serious adverse device effects

An adverse device effect is any untoward and unintended response to a medical device. This includes any event resulting from insufficiencies or inadequacies in the instructions for use or the deployment of the device as well as any event that is result of a user error.

An adverse device effect is classified as serious if it has resulted in any of the consequences characteristic of a serious adverse event or that might have led to any of these consequences if suitable action had not been taken or intervention had not been made or if circumstances had been less opportune.

6.3 Expected adverse events

As with any coronary intervention, there are possible risks and the possibility of developing complications:

Death, myocardial infarction, emergent repeat hospital intervention, bailout stenting, emergency CABG, aneurysm, pseudo aneurysm, distal embolization, air embolization, vessel spasm, fistulisation, haemorrhage, infection, restenosis of the vessel (greater than 50% obstruction), rupture of the vessel, stent migration/embolization, thrombosis (acute, subacute or late), failure to deliver the stent(s) to the intended site, occlusion, dissection, arrhythmias, prolonged angina, cardiac tamponade, perforation, hypotension, haematoma, allergic reaction, fever, stroke, renal failure, balloon rupture, dissection of non-treated site, retained pieces of dilatation catheter, stent compression, angina pectoris, bleeding complications, endocarditis, hemodynamic changes to flow, injury of the coronary artery, ischemia, palpitations, pulse arrhythmia, pyrogenic reaction, sepsis/infection, unstable angina, ventricular fibrillation.

However, the additional risk related to additional investigation-related measurements (measurement of the IMR and LVEDP), is extremely low.

7. WITHDRAWAL FROM STUDY/STUDY DISCONTINUATION

A patient's participation in the trial is voluntary and the patient may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the patient is otherwise entitled.

Drop outs are defined as patients:

- who decided not to continue the study/not to perform the follow-up visits for whatever reason
- who died

When a participant is withdrawn from the study, follow-up by phone call will be continued, unless the patient does not provide consent to follow-up. However, all data collected prior to discontinuation from the study will be retained in the database.

8. DATA COLLECTION

8.1 Data collection

The investigator or an individual designated by the investigator is responsible for recording all data from the study in patient's medical history and Case Report Form.

8.2 Protocol deviations

A protocol deviation is defined as an event where the clinical investigator or site personnel did not conduct the study according to the clinical investigation plan.

8.3 Record retention by study site

All study records and reports will remain in file at the study sites for a minimum of 25 years after completion of the study and will further be retained in accordance with local applicable law and guidelines.

8.4 Monitoring of data to source documents

A Case Report Form will be completed at the study site for each subject enrolled in the study. The case report forms will contain a record of the subject's eligibility to enter the study, medical history, pre procedure work up and clinical laboratory results, concurrent medications, plus a record of all devices used during the procedure, all procedural complications and adverse events, discharge, follow up, and any non-scheduled visit involving a late adverse event requiring hospitalization or additional procedures. The case report forms will be monitored at the site to verify source data accuracy and provide quality assurance.

9. STATISTICS AND ANALYSIS

9.1 Sample Size

The sample size for the ***primary objective*** (change in FFR after aortic valve intervention) is based on previous measurements of FFR in patients with aortic stenosis, where a standard deviation of the data was reported.[8,14,23] An expected mean FFR of 0.82 with a standard deviation of 0.08 was used in the calculation. Fifty eight (58) patients with pre- and post-interventions catheterizations will be sufficient to detect a reduction in the FFR from 0.82 to 0.79 with an alpha level of 0.05 and power of 0.80. A total sample of 100 patients with baseline measurements of which 80 patients will undergo (T)AVR without revascularisation (20% will undergo revascularisation) and in this way are eligible for pre-and post-intervention catheterization will allow for a 25 % drop-out during the follow-up phase of the study. Missing values will predominantly be at random. Therefore, the primary endpoint will be assessed using complete case analysis. To account for missing values, the number of patients will be increased from 58 to 80 (since missing data will be excluded from analysis).

For the ***secondary objective*** (comparison of FFR versus RFR): approximately 10-20% will have left main CAD, triple vessel disease, prior myocardial infarction or tandem lesions and will not be eligible for SPECT. One Hundred patients with baseline measurements will allow a 20% drop-out to end up with 80 patients with both baseline SPECT and invasive measurements. Yamanaka *et al*.[1] found that a sample of 80 patients was necessary to determine the optimal cutoff value of iFR for indicating myocardial ischemia. Since RFR and iFR have an extremely high correlation (0.99),[7] the same number of patients (80) is expected to achieve an area under the ROC curve of 0.70 with 0.90% power at a significance level of 0.05 in a 2-sided test.

9.2 Statistical analysis

All quantitative variables will be tested for normal distribution according to the Kolmogorov-Smirnov test. Statistical analyses will be performed via the SPSS statistical software package,

applying a significance level of ≤0.05. Continuous variables will be expressed as mean ± SD. Comparisons will be done with paired t-, Wilcoxon rank signed, Mann-Whitney U- or Kruskal-Wallis test when appropriate. The chi-square test will be applied for categorical variables and results will be expressed as n (%). Analysis will be performed to compare the intra-coronary measurements (FFR, RFR, IMR) prior to intervention (group 1) versus 6 months after intervention (group 2). Descriptive statistics (mean/median, standard deviation and percentiles for continuous data; percentage for categorical data) and graphical representation (boxplots, histograms and density plots) will be used to compare both groups' characteristics. Intra-coronary measurements in both groups will then be compared and tested using statistical tests (paired t-, Wilcoxon rank signed test). The correlation between FFR and RFR will be analyzed using Pearson correlation coefficient. The diagnostic performance of RFR in indicating SPECT myocardial ischemia will be evaluated using receiver operating characteristic (ROC) curve analysis.

10. REGULATORY REQUIREMENTS

10.1 Institutional Review Board and Ethics Committee: IRB/IEC

The clinical investigation plan as well as other study documents required by the ethics committee will be submitted for getting approval from the IRB/IEC.

10.2 Insurance

In accordance with the Belgian Law relating to experiments on human persons dated May 7, 2004, Sponsor shall assume, even without fault, the responsibility of any damages incurred by a Study subject and linked directly or indirectly to the participation to the study, and shall provide compensation therefore through its insurance.

10.3 Confidentiality

Patient confidentiality will be maintained throughout the study. It will be ensured that the information can always be tracked back to the source data, if required. Data relating to the study might be made available to regulatory authorities) preconditioned the data are treated confidentially and that the patient's privacy is guaranteed.

10.4 Publication Policy

The study results will be disseminated, valorized and published via abstracts and publications in high-index, peer-reviewed journals. Also, the results will be presented at several international (EuroPCR, TCT, etc.) and national (Belgian Society of Cardiology (BSC), Belgian Working Group of Interventional Cardiology (BWGIC) congresses and symposia.

1. Intellectual Property

Any Intellectual Property conceived or reduced to practice under this Agreement solely by Principal Investigator, Institution' (UZ Leuven, Belgium) or local study site' employees shall be owned by the Institution ("Institution Intellectual Property"); provided, however, no Institution Intellectual Property shall include Confidential Information provided by the local study sites. To the extent permitted by the policy of a medical-professional journal, medical institution, educational institution, or medical association relating to any publication or presentation of study data or results hereunder, ownership of the copyright in any publication, abstract, or presentation approved under this section will belong to the Institution (UZ Leuven).

2. Publication

The Principal Investigator shall have the right, and shall use reasonable efforts, to publish or otherwise publicly disclose study data and results and shall have the final authority to determine the scope and content of any publications originating from the study provided that before publishing, Institution shall submit copies of any proposed publication or presentation to all co-investigators for review. Institution shall in good faith consider any comments provided by any co-investigator and shall delete any Confidential Information identified prior to submission.

11. DEFINITIONS

Coronary angiography (CA): an invasive procedure usually carried out through the radial or femoral artery. Once a sheath has been positioned in a peripheral artery, catheters are used to inject an iodine-based contrast into the coronary arteries in order to permit their visualization.

Right and left heart catheterization (RHC): an invasive procedure that involves catheterization of the right heart and pulmonary arteries via a peripheral (usually jugular or femoral) vein and the left heart as per coronary angiography often with measurement of left ventricular pressure. Often performed at the same time as a coronary angiogram.

Cardiac catheterization: a broad term covering both coronary angiography and right and left heart catheterization.

Percutaneous coronary intervention (PCI): a percutaneous procedure (as per coronary angiography) with an intervention aimed at restoring normal flow in one/more of the coronary arteries.

Severe aortic stenosis: detailed assessment of severe aortic stenosis can be found in the ECS guideline for Valvular Heart Disease.[2] In patients with normal left ventricular function, severe AS is characterized by a mean gradient over the aortic valve of at least 40mmHg or an estimated valve area of less than 1.0cm^2.

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