AZIENDA OSPEDALIERA UNIVERSITARIA INTEGRATA
VERONA
(D.Lgs. n. 517/1999 - Art. 3 L.R. Veneto n. 18/2009)
DIPARTIMENTO AD ATTIVITÀ INTEGRATA CARDIO-VASCOLARE E TORACICO
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Version 1, 01/03/2025

Verona coronary physiology interventional registry

ACRONYM: VR-CP

Ethical committee authorization code: 618-CET

Sponsor: Azienda Ospedaliera Universitaria Integrata Verona – UOC Cardiologia

Principal investigator: Roberto Scarsini, MD PhD

Co-investigators:

- Simone Fezzi MD
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- Flavio Ribichini MD

Version number 1 of March 1st 2025

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SIGNATURE PAGE, APPROVAL OF STUDY PROTOCOL

I, the undersigned, have read and understand the protocol and agree that it contains all necessary information for conducting the study for my position in the study and I agree to conduct the trial as set out in this study protocol, the current version of the World Medical Declaration of Helsinki, ICH-GCP guidelines and the local legally applicable requirements.

Study Principal Investigator
Roberto Scarsini

Date: Verona, 15/09/2025

SUMMARY

Abbreviation

AE: adverse event

ACS: acute coronary syndrome

CAD: coronary artery disease

CCS: chronic coronary syndrome

DES: drug-eluting stent

eCRF: electronic clinical report form

FCA: functional coronary angiography

FFR: fractional flow reserve

IVUS: intravascular ultrasound

LDL: low density lipoprotein

VLDL: very low-density lipoprotein

MI: myocardial infarction

MLA: minimum lumen area

OCT: optical coherence tomography

PCI: percutaneous coronary intervention

SAE: serious adverse event

SAQ: Seattle angina questionnaire

TCFA: thin cap fibroatheroma

TVR: target vessel revascularization

VOCE: vessel-oriented cardiac adverse events

1. SYNOPSY

Study title	***Verona coronary physiology interventional registry (VR-CP)***
Protocol version	V1
Date	March, 1st 2025
Study sponsor	Azienda Ospedaliera Universitaria Integrata di Verona
Study principal investigator	Roberto Scarsini MD PhD
Patient population	The observational Verona Coronary Physiology Interventional Registry (VR-CP) study enrols patients with suspected coronary artery disease who underwent coronary angiography with functional assessment (coronary physiology).
Primary objective	The primary objective is to assess the functional characteristics of coronary lesions documented during coronary angiography and correlate them with the risk of cardiovascular events during follow-up.
Secondary objective	- To examine the prognostic value of physiological parameters (FFR and angiography-derived FFR). - To analyze the clinical, demographic, and angiographic characteristics of patients with coronary lesions assessed by coronary physiology parameters, in order to identify high-risk subgroups. - To correlate coronary physiology values with angina symptoms. - To compare invasive FFR with angiography-derived FFR. - To compare clinical outcomes between patients managed conservatively and those undergoing invasive interventions.
Study design	The VR-CP study is a single-center observational study enrolling consecutive patients with coronary stenoses documented during coronary angiography and functionally assessed with coronary physiology (fractional flow reserve [FFR] or angiography-derived FFR) performed for suspected ischemic heart disease. Follow-up is conducted, after obtaining informed consent, through telephone contact and/or outpatient visits. Adverse events are adjudicated by an independent clinical events committee, blinded to clinical data.
Data source	The selection of eligible patients is extracted from registries and the analysis of coronary angiographic studies performed from January 2010 to December 2024.
Sample size	Enrollment will include approximately 1,000 patients evaluated with FFR or angiography-derived FFR. This sample size will allow for subgroup analyses stratifying lesions by functional severity based on FFR values.
Inclusion criteria	- Coronary angiography with functional assessment by FFR or angiography-derived FFR (index procedure) - Provision of written informed consent - Age ≥18 years old at the time of the index procedure

Exclusion Criteria	- Inability to provide informed consent to participate in the study
Informed Consent	Patients identified as eligible for the protocol will be invited to provide informed consent for participation in the retrospective study through contact by telephone, e-mail, mail, or during an outpatient visit.
Study Procedures	Once eligibility for the protocol is confirmed, informed consent is requested and obtained. After enrolment, the angiographic images from the index coronary angiography are analysed by the investigators. Long-term follow-up is performed by monitoring the clinical status and recording major adverse cardiac events through review of medical records, outpatient visits, or telephone contacts.
Primary Endpoint	Composite of vessel-oriented composite endpoint (VOCE), including target vessel revascularization (TVR), target vessel-related myocardial infarction (MI), and cardiovascular death.
Secondary Endpoints	- Individual components of the primary endpoint - Hospitalization for unstable angina - Angina status (Seattle Angina Questionnaire) - Number of prescribed antianginal medications
Study Duration	Patients are retrospectively enrolled from January 2010 to December 2024. Clinical contact (medical records, outpatient visits, telephone communication) is periodically collected recording longitudinal data at 12-month intervals.
Clinical Event Adjudication Committee	The following events are adjudicated by an independent committee blinded to clinical data: TVR, target vessel-related MI, cardiovascular death, and hospitalization for unstable angina.

2. INTRODUCTION

Integrated assessment of lesion hemodynamic impact and high-risk plaque features: an unmet clinical need.

Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality in developed countries [1]. Coronary angiography remains the gold standard for diagnosing CAD, however, its ability to differentiate ischemic from non-ischemic lesions is limited [2]. Relying solely on the anatomical evaluation through coronary angiography fails to reveal the hemodynamic significance of epicardial CAD [3]. The 2-dimensional representation of the arterial lesion provided by angiography is limited in distinguishing intermediate lesions that require stenting from those that simply need appropriate medical therapy [4]. In the current era of drug-eluting stents (DES), when percutaneous coronary revascularization is achieved with high success rates, low complication rates, and excellent long-term patency, it might be tempting to treat all suspect lesions with DES implantation [4]. However, procedural complications associated with percutaneous coronary intervention (PCI), the inherent risk of restenosis, and late stent thrombosis still exist.
Histopathological studies have demonstrated that angiographic evidence of stenosis is usually not detected until the plaque's cross-sectional area approaches 40% to 50% of the vessel's total cross-sectional area. The outer wall of the artery, encompassed by the external elastic membrane, enlarges to accommodate the growing plaque. This process of compensatory dilatation seems to be limited. When the plaque area exceeds 40% to 50% of the external elastic membrane area, the plaque begins to encroach upon the lumen. Only at this point the angiogram might reveal minimal luminal narrowing [4–7].
In this respect, coronary physiology, which consider the functional severity of coronary stenosis, outperforms the traditional diagnostic approach based solely on morphometric assessment.

Consequently, invasive wire-based solutions for physiological assessment were developed to identify ischemia-producing epicardial coronary stenoses [3, 8]. Current European guidelines emphasize the importance of performing physiological assessment for all intermediate coronary artery stenoses, including left main stenosis, side branch stenosis and serial stenoses, when functional information from non-invasive tests is lacking [9]. Moreover, in patients with multivessel CAD, PCI guided by fractional flow reserve (FFR) results in an improved outcome irrespective of available non-invasive functional test results [9]. The development of FFR and its use in guiding percutaneous intervention, has led to improved patient outcomes compared with angiography guidance alone [10]. New developments in wire-free functional coronary assessment derived from the angiogram, globally termed as functional coronary angiography (FCA), may contribute to a more widespread use of physiology to diagnose the cause of ischemia and to plan and guide coronary revascularization [11–16]. These systems are based on vessel lumen reconstructions from coronary angiography, with the aim of providing FFR-like estimates of stenosis severity, whilst not requiring pharmacological agents or intracoronary instrumentation. Different software approaches to FFR estimation derived from coronary angiography have been developed. Several studies demonstrated the good correlation between wire-based and Angiography-derived FFR with a cut-off of 0.80. In a recent meta-analysis of 16 studies, 819 patients and 969 vessels with paired FFR and Angiography-derived FFR were included. Authors found an overall satisfactory agreement (mean difference 0.009 ± 0.068, I2 = 39.6) of angiography- and wire- derived FFR. The diagnostic performance was sensitivity 84% (95%CI: 77-90, I2 = 70.1), specificity 88% (95%CI: 84-91, I2 = 60.1); positive predictive value 80% (95%CI: 76-85, I2 = 33.4), and negative predictive value 95% (95%CI: 93-96, I2 = 75.9) [15]. Several non-inferiority trials are ongoing to provide the data answer regarding Angiography-derived FFR clinical equivalence in comparison with wire-based FFR

(Tab.I). Angiography-derived FFR analyzability depends on quality of angiography, and it is feasible in around 80% of the cases.

The Vulnerable Plaque: Pathophysiology of Coronary Artery Disease Progression

Acute coronary syndromes (ACS) primarily result from the rupture or erosion of a vulnerable plaque [17]. After the first acute event, a residual risk of recurrent coronary events persists despite optimal medical therapy. Target lesion–related events mainly drive this risk during the first year. Thereafter, the risk stems from other lesions, which in most cases were only angiographically mild during the index procedure and were not considered at risk of rupture on initial coronary angiography, as demonstrated in several retrospective studies [17, 18].

Indeed, even when non-obstructive and non–flow limiting on angiographic and/or functional assessment, a coronary lesion may harbor vulnerable features not detectable by angiography that increase the risk of rupture [19].

The concept of “vulnerable plaque” was first introduced in 1989 by Muller et al., referring to plaques associated with an increased risk of destabilization, rupture, and subsequent vessel occlusion. They observed that, in most cases, the culprit vessel displayed only mild stenosis, not angiographically critical, and that thrombosis was the main mechanism of vessel occlusion. Since then, several studies have focused on defining, detecting, and analyzing plaque vulnerability[20]

In 2003, Naghavi et al. published the first consensus document to standardize definitions of vulnerable plaques, described as “all plaques prone to thrombosis and with a high likelihood of rapid progression, thereby becoming culprit plaques”. Five major vulnerability criteria were identified: 1) a thin fibrous cap with a large lipid core; 2) active inflammation within the plaque

(e.g., macrophage infiltration); 3) endothelial denudation leading to superficial platelet aggregation (e.g., plaque erosion); 4) a fissured plaque (mainly indicating recent rupture); 5) diameter stenosis >90%. Minor criteria included the presence of superficial calcific nodules, shiny yellow plaque on angioscopy, intraplaque hemorrhage, endothelial dysfunction, or outward remodeling. Multiple mechanisms contribute to plaque development and destabilization. Accumulation of low- and very-low-density lipoproteins (LDL and VLDL), lipoprotein(a)-mediated inflammation, increased expression of adhesion molecules on endothelial cells, and active inflammation are key drivers of this process. Persistent elevation of circulating lipid levels promotes the formation of vulnerable plaques with a large necrotic core and a thin fibrous cap [20–22].

If the thin fibrous cap ruptures, the lipid-rich plaque releases its thrombogenic content into the bloodstream, leading to the formation of an occlusive (or sub-occlusive) intraluminal thrombus responsible for ACS. Thus, the thickness and quality of the fibrous cap influence the risk of rupture, although the triggering factors remain incompletely understood. Proposed triggers include changes in atherosclerotic plaque components, blood influx through the vasa vasorum, and the functional integrity of the arterial intima. Degradation of the extracellular matrix in the arterial wall by matrix-degrading enzymes (e.g., matrix metalloproteinases) may further contribute. Perivascular adipose tissue (PVAT) may also be involved. Production of reactive oxygen species (ROS) and expression of intercellular and vascular adhesion molecules by endothelial cells promote active inflammation, activation of vascular smooth muscle cells, and increased release of proinflammatory cytokines and ROS. These factors activate PVAT, which responds by releasing proinflammatory adipocytokines, lipid mediators, and ROS, thus perpetuating a vicious cycle of active inflammation and atherosclerosis progression [19, 23].

The PROSPECT study, the first prospective natural-history investigation correlating plaque features with subsequent cardiovascular events, included nearly 700 patients undergoing percutaneous

coronary intervention for ACS who also underwent intravascular ultrasound (IVUS). The aim was to assess the incidence of major adverse cardiovascular events related to both the culprit lesion and an additional non-culprit lesion. It was observed that 20.4% of patients experienced an event at 3-year follow-up, equally attributable to culprit and non-culprit lesions, with most non-culprit event-related lesions being angiographically mild at baseline (mean diameter stenosis 32.3 ± 20.6%). Gray-scale and radiofrequency IVUS analysis showed that the presence of a thin-cap fibroatheroma (TCFA), together with a plaque burden >70% and a minimal luminal area (MLA) <4.0 mm², was a strong independent predictor for non-culprit lesions. Vulnerable non-culprit plaques are common in patients with multivessel disease. A secondary analysis of the COMPLETE trial showed that nearly 50% of patients with ST-segment elevation myocardial infarction (STEMI) had at least one non-culprit obstructive TCFA. Moreover, obstructive lesions (>70% diameter stenosis on visual assessment) more often contained vulnerability features than non-obstructive lesions. Another interesting study by Zhao et al. showed that, among STEMI patients who underwent optimal optical coherence tomography (OCT) imaging of all three coronary arteries, those with plaque progression at 12 months (defined as a ≥0.4 mm reduction in minimal luminal diameter) had more high-risk plaque features (47.4% vs. 33.3%; p = 0.036) and more non-culprit plaque ruptures (25.6% vs. 4.5%, p = 0.036). [18]

The Vulnerable Plaque and the Vulnerable Patient: A Targeted Therapeutic Approach

Atherosclerosis progresses through repeated cycles of plaque rupture and healing, driven by persistent active inflammation, ultimately leading to progressive luminal narrowing. This

phenomenon is particularly pronounced in vulnerable plaques compared to stable lesions. Since inflammation promotes atherosclerosis progression, plaque destabilization, and a prothrombotic state, targeted therapeutic strategies aimed at reducing both local and systemic inflammation have been proposed and tested to improve cardiovascular outcomes.[22]

Ongoing studies are evaluating preventive strategies based on intensification of pharmacologic therapy in patients at high risk of cardiovascular events, or a combination of medical therapy with prophylactic revascularization of vulnerable plaques.[22]

With regard to medical therapy, randomized clinical trials have demonstrated that intensive lipid-lowering therapy stabilizes vulnerable plaques, either with high-dose statin therapy alone or in combination with a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor [19]

As for prophylactic revascularization of vulnerable lesions, robust evidence indicates that in chronic coronary syndromes (CCS), this strategy has not significantly improved long-term outcomes. Conversely, in patients with STEMI and multivessel disease—who exhibit a higher prevalence of vulnerable plaques than those with CCS—elective treatment of non-culprit obstructive lesions has been shown to reduce the 5-year incidence of myocardial infarction and future acute coronary syndromes in the COMPLETE trial [19, 24]. Following stenting, neointimal hyperplasia generates a new fibrous cap, thickening the pre-existing one. Moreover, the use of everolimus-eluting stents has been shown in animal models to reduce plaque macrophage content. Large-scale randomized clinical trials are underway to determine whether focal therapy of imaging-identified vulnerable plaques can improve overall patient outcomes. Recently published results from the PREVENT trial—randomizing more than 1,500 patients with CCS or ACS in a 1:1 ratio to medical therapy alone or medical therapy plus revascularization (using bioresorbable vascular scaffolds or second-generation everolimus-eluting stents) of non–flow-limiting, non-culprit vulnerable plaques identified by IVUS, radiofrequency IVUS, or OCT—appear to support the interventional strategy.

At two years, the composite endpoint of cardiac death, target vessel myocardial infarction, ischemia-driven target vessel revascularization, or hospitalization for unstable or progressive angina was significantly lower in the PCI group (absolute difference –3.0%, 95% CI –4.4 to –1.8, p=0.0003) [25].

3. STUDY DESIGN

The Verona Coronary Physiology Interventional Registry (VR-CP) study is a single-center, single-arm, observational study with blinded event adjudication. VR-CP enrolls patients who underwent coronary angiography for suspected ischemic heart disease, in whom at least one coronary lesion was identified and functionally assessed by coronary physiology testing (fractional flow reserve [FFR] or angiography-derived FFR).

Eligible patients are identified through registry data and review of coronary angiographic studies performed from January 2010 to December 2024. Patients meeting inclusion criteria are invited to provide written informed consent to participate in the observational study. Recruitment may occur through telephone, mail, email, or during outpatient visits. All study data are recorded in an anonymized electronic case report form (eCRF).

The follow-up involves periodic recording of the clinical status and reporting of any adverse events through review of medical records, outpatient visits, or telephone communications, in order to ensure longitudinal data collection at 12-month intervals.

Adverse events are adjudicated by an independent Clinical Event Committee (CEC) blinded to all patient clinical data. Investigators will provide the CEC with anonymized clinical documentation relating to the reported adverse event. The CEC will adjudicate events that are part of the study's primary and secondary endpoints. Event definitions and adjudication rules are established by the

Principal Investigators and recorded in the Adjudication Committee Charter. The adjudication outcomes determined by the CEC will be used for the final statistical analysis.

CEC members will be appointed by the Principal Investigator at the time of study activation. They will be selected among experienced cardiologists with no access to the clinical documentation of enrolled patients.

4. STUDY OBJECTIVES

Primary Objective

The primary objective is to identify a risk profile for vessel-oriented composite endpoint (VOCE) associated with the functional characteristics of coronary lesions documented during coronary angiography.

Secondary Objectives

Secondary objectives include:

- Assessing the prognostic value of physiological parameters (invasive FFR and angiography-derived FFR).
- Analysing the clinical, demographic, and angiographic characteristics of patients with coronary lesions evaluated by coronary physiology to identify high-risk subgroups.
- Investigating the correlation between coronary physiology values and angina symptoms.
- Comparing invasive FFR with angiography-derived FFR.
- Comparing clinical outcomes between patients managed conservatively and those undergoing invasive interventions.

5. POPULATION

Inclusion Criteria

- Previous coronary angiography with assessment of FFR and/or angiography-derived FFR.
- Age ≥ 18 years old*
- Provision of written informed consent.

* At the time of the index coronary angiography.

Exclusion Criteria

- Inability to provide informed consent to participate in the study.
- Age < 18 years old*

* At the time of the index coronary angiography.

Vulnerable Populations

The following subjects will not be eligible for inclusion:

- Pregnant women
- Women who are breastfeeding

Criteria for Early Withdrawal from the Study

Subject's request to withdraw participation

6. DESCRIPTION OF THE DISEASE IN THE STUDY

Ischemic Heart Disease and General Information on Coronary Angiography

The selection of eligible patients is based on the review of hospital registries and the analysis of coronary angiography procedures performed from January 2010 to December 2024.

Participants with suspected CAD undergo coronary angiography as part of the standard diagnostic and therapeutic pathway, in accordance with current guidelines for the management of ischemic heart disease. A patient is considered eligible if at least one mild or intermediate coronary stenosis is identified on angiographic assessment and subsequently evaluated with a coronary physiology test (fractional flow reserve [FFR] or angiography-derived FFR). These functional assessments guide the operator's decision to proceed with or defer vessel revascularization.

If a physiological evaluation of CAD is performed, the corresponding data — including FFR and/or angiography-derived FFR — are collected and recorded. The initial angiogram of all patients will be stored, anonymized, and kept locally for functional analysis (acceptable formats include CD, DVD, or digital files).

7. ENDPOINTS

Primary Endpoint

The primary endpoint is the composite risk of vessel-oriented composite endpoint (VOCE), including target vessel revascularization (TVR), vessel-related myocardial infarction (MI), and cardiovascular death.

Secondary Endpoint

- Individual components of the primary endpoint.
- Hospitalization for unstable angina.
- Angina status assessed using the Seattle Angina Questionnaire (SAQ). The SAQ will be used to evaluate patients' quality of life and the impact of angina symptoms over time. Scores from the main subscales (physical limitation, angina stability, angina frequency, treatment perception, and overall quality of life) will be analysed to correlate symptom severity with coronary physiology parameters (e.g., FFR/angiography-derived FFR).
- Number of prescribed antianginal drugs. The number of prescribed antianginal medications will be monitored to assess the intensity of pharmacological treatment required during follow-up. This parameter will serve as a proxy for residual disease severity and will allow comparison between patients managed conservatively and those treated with invasive interventions.

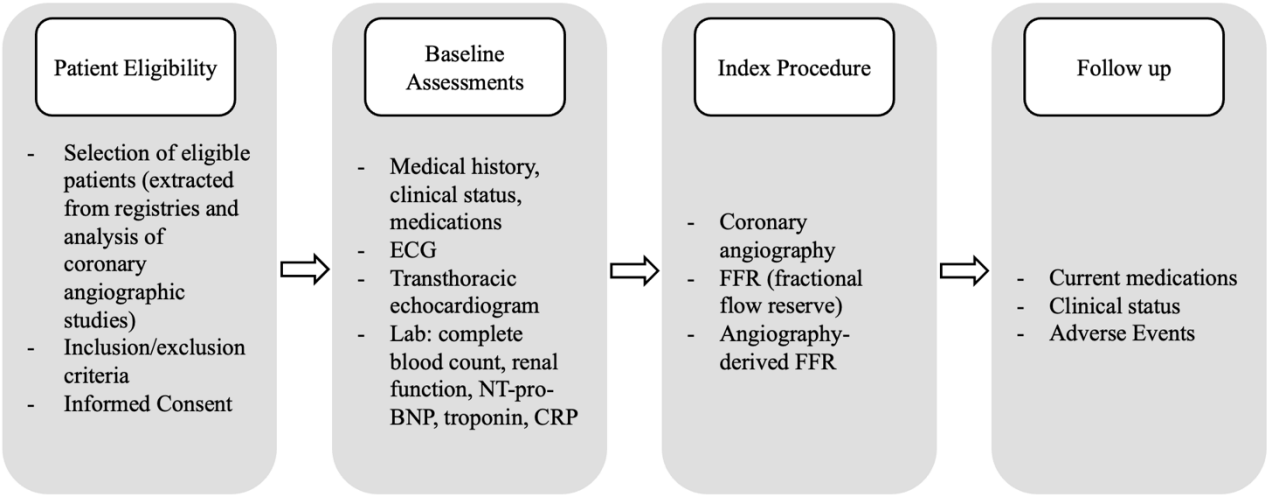
8. PLANNED PROCEDURES AND DATA COLLECTION

The VR-CP study is a retrospective observational study enrolling consecutive patients who underwent coronary angiography for suspected ischemic heart disease and were found on angiographic examination to have coronary lesions evaluated using coronary physiology testing (FFR or angiography-derived FFR). These physiology assessments guided the decision to proceed with or defer target vessel revascularization.

Regardless of whether the lesion was treated invasively or managed conservatively, patients' clinical status and any adverse events are periodically recorded.

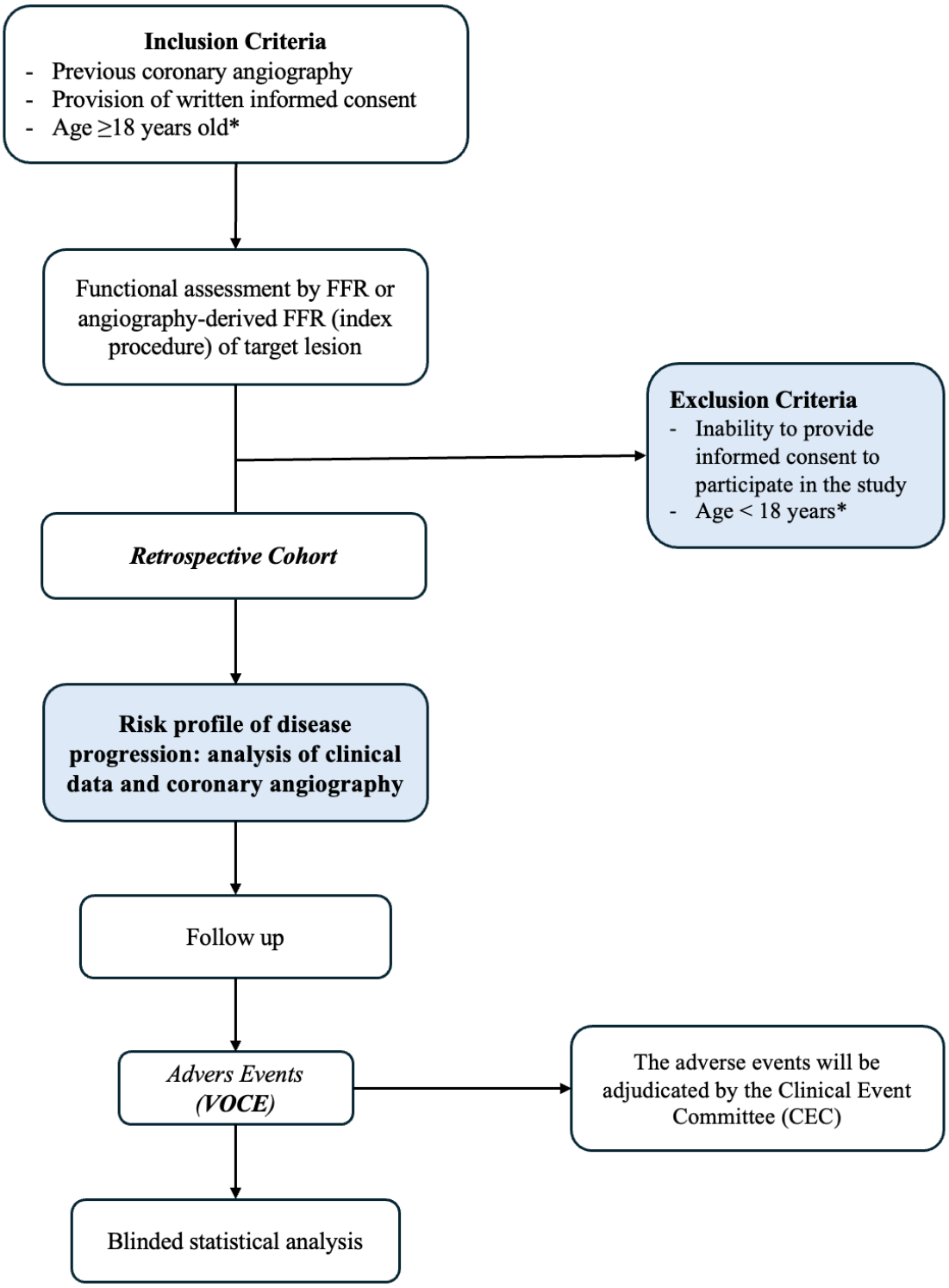
This retrospective follow-up is conducted through the collection of information from medical records, direct contact during outpatient visits, or via telephone communications, in order to ensure longitudinal data collection at 12-month intervals.

Fig. 1
Summary of Activities Performed



Flowchart

Fig.2 Retrospective Observational Study Design



*at the time of index procedure

DATA MANAGEMENT AND PROCEDURES TO ENSURE DATA CONFIDENTIALITY

The Sponsor commits to comply with Privacy Laws (as defined below), including provisions relating to security measures and confidentiality. The Sponsor guarantees, for itself and the Investigator, full awareness of all obligations arising from any applicable regulations concerning professional medical confidentiality and the protection of patients' personal data, including but not limited to EU Regulation 2016/679, the Italian Privacy Code (Legislative Decree 196/03, as amended), the provisions, guidelines, and current general authorizations issued by the Italian Data Protection Authority (collectively "Privacy Laws"). The Sponsor ensures that all personnel involved in the conduct of the study comply with Privacy Laws and the Sponsor's instructions regarding the protection of personal data, including security and confidentiality aspects. This obligation includes, for example: (i) providing the patient with a complete and lawful privacy notice (in particular, EU Regulation 2016/679, Legislative Decree 196/2003 as amended, and the aforementioned guidelines dated July 24, 2008); (ii) obtaining the patient's written informed consent before participation in the study; (iii) respecting the privacy rights of each data subject as established by applicable Privacy Laws; (iv) adopting all appropriate physical, logical, organizational, technical, and IT measures in compliance with applicable Privacy Laws. Due to the particular sensitivity of the data processed in the study, specific technical measures have been adopted to increase data security, without prejudice to any other minimum measures. These relate especially to the recording of data on persons involved in the study at the investigative sites, whether electronic or paper-based, their electronic transfer to a centralized database held by the Sponsor or third parties performing data validation and statistical processing on behalf of the Sponsor, and the management of this database. The Sponsor has taken appropriate measures to protect the recorded data from risks of unauthorized access, theft, or partial or total loss of paper documents, storage media, or fixed or portable processing systems.

Access to computers storing the data requires a username and password. Furthermore, access to the database for data entry sessions is protected by username and password. To ensure data confidentiality and to prevent data manipulation or loss, the following precautions are taken: Data access is restricted to authorized members only. The network is protected by a firewall. Internet connections are encrypted with a digital certificate (SSL technology). The database is hosted on a password-protected server, with passwords changed periodically. Database access is password protected and limited to responsible personnel at the center. Periodic backups are performed.

Paper materials related to clinical assessments will be stored in locked cabinets, with keys held only by persons authorized by the study management. The Sponsor has adopted secure communication protocols based on cryptographic standards for the electronic transmission of data collected from investigative sites to the centralized database maintained by the Sponsor or third parties performing subsequent data validation and statistical processing. The Sponsor will require the adoption of secure communication protocols for the transmission of any paper documentation. Regarding the database, appropriate authentication and authorization systems are in place according to user roles and access needs, along with periodic procedures to verify the quality and consistency of authentication credentials and authorization profiles assigned to data handlers. The Investigator will adequately dissociate patient identifying data from study result data (e.g., by anonymizing results using randomly generated alphanumeric codes) so that only pseudonymized data are processed by the Sponsor.

The Sponsor will allow access to clinical data (including medical records) and any other information relevant to the study, always in compliance with applicable Privacy Laws and respecting data security and confidentiality measures. Within the limits provided by the information given to study patients, personal data will be accessible only to employees, collaborators, monitors,

and auditors of the Sponsor, and/or to competent authorities in the exercise of their functions. The Sponsor and Investigator commit to clearly and fully inform each study patient about the processing of their personal data before participation in the study, according to applicable Privacy Laws. Before data acquisition, the Investigator will provide appropriate information to each patient regarding the nature, purpose, outcomes, consequences, and risks of the study. Prior to patient enrollment, the Investigator or authorized delegate will obtain written informed consent from the patient for: (a) participation in the study; (b) communication of related confidential information; (c) processing of personal data; (d) transfer of documentation containing the patient's personal data, including sensitive health data, to the Sponsor and/or competent authorities and/or other institutions, including those outside the European Union, in accordance with legal requirements and applicable Privacy Laws. The Sponsor commits to retaining original paper documentation (e.g., informed consent forms) for at least 7 years in compliance with Legislative Decree 200/2007.

10. STUDY DURATION

Recruitment: Once eligibility for the protocol is confirmed, informed consent is requested and obtained, and the patient is then enrolled in the study.

Patient involvement: Enrollment includes patients undergoing coronary angiography from January 2010 to December 2024.

Follow up: Follow-up will be maintained until all patients have completed a clinical evaluation at 10 years.

11. SAMPLE SIZE CALCULATION

In contemporary clinical trials on coronary physiology indices, the composite adverse event rate at 12 months follow-up is relatively low, ranging from 6.1% in the SWEDEHEART-IFR study to 6.7% in the DEFINE-FLAIR study. Therefore, assuming a type I error of 0.05 and a statistical power of 80%, the study should include 1000–1200 patients to generate a sufficient number of evaluable events. This sample size will also allow for subgroup analyses stratifying lesions by functional severity based on FFR values.

12. STATISTICAL ANALYSIS

Continuous variables will be tested for normal distribution using the Kolmogorov-Smirnov test. Variables with a normal distribution will be presented as mean ± standard deviation and compared using t-tests and one-way ANOVA. Otherwise, median [interquartile range] will be used, and comparisons will be performed using the Mann-Whitney U and Kruskal-Wallis tests. Categorical variables will be summarized as absolute and relative frequencies (percentages) and compared using the χ^2 test. Correlations between variables will be evaluated using linear and logistic regression analyses. The level of statistical significance will be set at $\alpha = 0.05$. Kaplan-Meier curves will be plotted to describe event-free survival, and differences between groups will be tested using the log-rank test. The primary analysis will be based on events occurring during the entire follow-up period for each patient at database lock. Any confounding factors will be tested using Cox regression models. Variables with a p-value < 0.1 in univariate analysis will be included in a multivariate analysis to identify independent predictors. When appropriate, 95% confidence intervals will be calculated.

13. LIMITATIONS AND BIASES

The VR-CP retrospectively enrols patients who underwent coronary angiography documenting at least one mild to intermediate coronary lesion assessed by coronary physiology tests (FFR or angiography-derived FFR). The retrospective enrolment may not be fully representative of the general population or could be influenced by prior clinical decisions, thus introducing selection bias. To mitigate this issue, consecutive patients are enrolled, ensuring a more representative and generalizable sample. This approach minimizes the influence of selection bias, investigator bias, and temporal bias, improving the logistical simplicity and scientific rigor of the study. Our goal is to ensure that the sample reflects the typical patient population encountered in routine clinical practice.

Another limitation is that historical data might reflect medical practices or diagnostic techniques that are no longer routinely used or have evolved over time, limiting the generalizability of the results.

An independent Clinical Event Committee (CEC) evaluates all endpoints. The CEC members and management team are fully blinded to patients' identifying information. The CEC adjudicates events based on predefined definitions. Additional measures to prevent or minimize bias include the use of objective criteria for endpoint classification. Finally, unlike prospective randomized studies, this retrospective study does not include randomization, which may lead to uneven distribution of risk factors.

14. ADVERSE EVENTS

Definitions

Adverse Events (AEs) / Serious Adverse Events (SAEs) / Incidents:

Any undesirable medical occurrence in a clinical study during which a patient receives the study treatment, and which does not necessarily have a causal relationship with such treatment or with any device malfunction. An AE can therefore be an unintended and accidental manifestation, represented by a sign (including an abnormal laboratory result), a symptom, or a disease, whose onset is temporally associated with the study treatment or with any device malfunction, whether related or unrelated to the study treatment.

Serious Adverse Event (SAE):

Any undesirable medical event or device malfunction that results in one or more of the following outcomes:

- Death
- Life-threatening condition
- Hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Medically significant event or one that requires intervention to prevent any of the above outcomes. If an event meeting the SAE definition occurs after signing the informed consent form but before patient inclusion, it should not be reported as an SAE. Such an event may be reported in the appropriate section of the Case Report Form (CRF).

For medical devices and active implantable medical devices, an incident is defined as:

a) Any malfunction or deterioration in the characteristics or performance of a medical device/active implantable medical device, as well as any inadequacy in labeling or

instructions for use, which may have caused or contributed to death or serious deterioration in the health condition of a patient or user;

b) Any technical or medical reason related to the characteristics or performance of a medical device/active implantable medical device that leads to the systematic recall of devices of the same type by the manufacturer for reasons described in point (a). (Decree Legislative 24 February 1997, n. 46 – Implementation of Directive 93/42/EEC concerning medical devices, amended by D. Lgs. 25.01.2010, n. 37).

Monitoring of Adverse Events, Serious Adverse Events, and Incidents

As established by the Investigator, patients who experience Adverse Events (AEs), Serious Adverse Events (SAEs), or Incidents will be evaluated using relevant clinical assessments and laboratory analyses. Any actions taken and the outcomes of follow-up must be recorded in the patient's original documentation and in the appropriate sections of the Case Report Form (CRF).

To obtain evidence of adverse events throughout the entire case series, the patient will be interviewed and/or examined by the Investigator or an authorized delegate. Questions asked to patients regarding the possible occurrence of adverse events should be as non-specific as possible. However, since the center is responsible for conducting follow-up for all cases of AEs, specific questions regarding previously reported AEs, SAEs, and Incidents must be included concerning their resolution or continuation.

For treatment-related AEs/SAEs/Incidents, relevant clinical evaluations and laboratory analyses will be repeated until the event is fully resolved, stabilized, or its cause explained.

SAEs not related to treatment that remain unresolved at the completion of the case series must be followed for a maximum period of one month after the case series completion.

Reporting of Adverse Events, Serious Adverse Events, and Incidents

The reporting of an incident (whose definition is included within that of "serious adverse effect" used in clinical trials – see technical standard UNI EN ISO 14155-11) involving medical devices under post-marketing investigation must be carried out similarly to the reporting for medical devices used in routine clinical practice, through the completion of the Ministry's form for MD/IVD (Medical Devices/Active Implantable Medical Devices) — which requires indicating the study code number — or the form related to IVDs (In Vitro Diagnostics). A copy of the report must also be sent to the relevant Ethics Committee.

15. DEFINITIONS

Ischemia-driven target vessel revascularization (TVR)

All ischemia-driven revascularizations refer to the target lesion that led to enrollment into the study. Requires the following criteria:

- Ischemic symptoms consistent with CCS class ≥ 3 angina despite optimal medical therapy

AND

- PCI or CABG of either the target that led to enrollment into the study PLUS at least ONE of the following:
  - Positive functional study (Exercise or myocardial perfusion imaging or stress or dobutamine echo) demonstrating clear evidence of reversible ischemia corresponding to a stenosis in the target vessel;
  - New ischemic ECG changes (ST-segment depression ≥ 1 mm, ST-segment elevation ≥ 1 mm or T wave inversion ≥ 2 mm) at rest or with exertion in a distribution consistent with a stenosis in the target vessel;
  - FFR ≤0.80, iFR≤0.89, cFFR≤0.85, Angiography-derived FFR≤0.80 in the target vessel.

Vessel-related myocardial infarction (MI)

MI is defined according to the 4th universal definition of myocardial infarction. The leading symptom that initiates the diagnostic and therapeutic cascade in patients with suspected MI is chest pain. Based on the electrocardiogram (ECG), two groups of patients should be differentiated:
Patients with acute chest pain and persistent (>20 min) ST-segment elevation. This condition is termed ST-elevation ACS and generally reflects an acute total coronary occlusion. Most patients will ultimately develop an ST-elevation myocardial infarction (STEMI). The mainstay of treatment in these patients is immediate reperfusion by primary angioplasty or fibrinolytic therapy.
Patients with acute chest pain but no persistent ST-segment elevation. ECG changes may include transient ST-segment elevation, persistent or transient ST-segment depression, T-wave inversion, flat T waves or pseudo-normalization of T waves or the ECG may be normal. The clinical spectrum of NSTE-MI may range from patients free of symptoms at presentation to individuals with ongoing ischemia, electrical or hemodynamic instability or cardiac arrest.
The pathological correlate at the myocardial level is cardiomyocyte necrosis. A small proportion of patients may present with ongoing myocardial ischemia, characterized by one or more of the following: recurrent or ongoing chest pain, marked ST depression on 12-lead ECG, heart failure and hemodynamic or electrical instability. Due to the amount of myocardium in jeopardy and the risk of malignant ventricular arrhythmias, immediate coronary angiography and, if appropriate, revascularization are indicated. The clinical definition of MI denotes the presence of acute myocardial injury detected by abnormal cardiac biomarkers in the setting of evidence of acute myocardial ischaemia.
Any MI without a clearly identifiable culprit vessel is counted as target vessel related.

Cardiovascular death

Deaths are classified as cardiovascular, non-cardiovascular, or of undetermined cause according to ARC-2 criteria. Deaths of undetermined cause are classified as cardiovascular but as a separate subcategory of cardiovascular deaths to indicate this uncertainty. Deaths due to procedural complications or concomitant treatments are classified as cardiovascular. Only deaths due to a documented non-cardiovascular cause (e.g., cancer) are classified as non-cardiovascular. Cardiovascular deaths in patients with multivessel disease are assigned to each affected vessel.

16. ETHICAL ASPECTS AND REGULATORY STANDARDS

Good clinical practice

The latest version of the Declaration of Helsinki and the Oviedo Convention are the fundamental references for the clinical conduct of the study.

The study protocol is designed and it will be conducted to ensure the adherence to the principles and the procedures of Good Clinical Practice and to comply with the Italian laws, as described in the following documents and accepted with their signature by the investigators of the study:

- ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
- Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.
- D. L.vo n.211 del 24 giugno 2003.
- D. L.vo n.200 6 Novembre 2007.
- D.M. 21 Dicembre 2007.
- Determinazione AIFA 20 Marzo 2008.

All the essential clinical documents will be stored in order to prove the validity and the integrity of the data collected.

According to the responsibilities provided from the rules of Good Clinical Practice (d.lgs 211/2003) and the applicable laws and regulations in the field of data protection including the European Data Protection Regulation 2016/679, the Promotor of the study will process the personal data, exclusively with the aim of the realization of the study and for the purpose of pharmacovigilance or medical device vigilance.

17. DATA OWNERSHIP AND PUBLISHING AGREEMENTS

The data are exclusively owned by the Promotor of the study and by Azienda Ospedaliera Universitaria Integrata di Verona. Published data refers to the following protocol.

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