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Evaluation of Microvascular Rarefaction in Vascular Cognitive Impairment and Heart Failure (CRUCIAL): Study Protocol for an Observational Study

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Keywords
Microvascular disease · Microvascular rarefaction · Heart failure with preserved ejection fraction · Vascular cognitive impairment

Abstract
Introduction: Microvascular rarefaction, the functional reduction in perfused microvessels and structural reduction of microvascular density, seems to be an important mechanism in the pathophysiology of small blood vessel-related disorders including vascular cognitive impairment (VCI) due to cerebral small vessel disease and heart failure with preserved ejection fraction (HFpEF). Both diseases share common risk factors including hypertension, diabetes mellitus, obesity, and ageing; in turn, these comorbidities are associated with microvascular rarefaction. Our consortium aims to investigate novel non-invasive tools to quantify microvascular health and rarefaction in both organs, as well as surrogate biomarkers for cerebral and/or cardiac rarefaction (via sublingual capillary health, vascular density of the retina, and RNA content of circulating extracellular vesicles), and to determine whether microvascular density relates to disease severity. ***Methods:*** The clinical research program of CRUCIAL consists of four observational cohort studies. We aim to

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recruit 75 VCI patients, 60 HFpEF patients, 60 patients with severe aortic stenosis (AS) undergoing surgical aortic valve replacement as a pressure overload HFpEF model, and 200 elderly participants with mixed comorbidities to serve as controls. Data collected will include medical history, physical examination, cognitive testing, advanced brain and cardiac MRI, ECG, echocardiography, sublingual capillary health, optical coherence tomography angiography (OCTa), extracellular vesicles RNA analysis, and myocardial remodelling-related serum biomarkers. The AS cohort undergoing surgery will also have myocardial biopsy for histological microvascular assessment. ***Discussion:*** CRUCIAL will examine the pathophysiological role of microvascular rarefaction in VCI and HFpEF using advanced brain and cardiac MRI techniques. Furthermore, we will investigate surrogate biomarkers for non-invasive, faster, easier, and cheaper assessment of microvascular density since these are more likely to be disseminated into widespread clinical practice. If microvascular rarefaction is an early marker of developing small vessel diseases, then measuring rarefaction may allow preclinical diagnosis, with implications for screening, risk stratification, and prevention. Further knowledge of the relevance of microvascular rarefaction and its underlying mechanisms may provide new avenues for research and therapeutic targets.

Introduction

Vascular cognitive impairment (VCI) and heart failure (HF) are major causes of morbidity and mortality worldwide. VCI refers to cognitive impairment due to vascular diseases of the brain and can range from mild cognitive impairment to frank dementia [1]. VCI is the second most common form of dementia contributing to between 20 and 50% of cases [2–4], with a significant socioeconomic burden [5]. HF is highly prevalent, with 64 million cases worldwide [6] and an estimated 5.2 million HF hospitalizations annually in Western Europe [7]. When stratified by left ventricular ejection fraction (LVEF), 50% of HF hospitalizations occur in those with LVEF >50%, termed HF with preserved ejection fraction (HFpEF) [8]. Both VCI and HFpEF are associated with increasing age, arterial hypertension, chronic kidney disease, obesity, and diabetes mellitus (DM). With an increasingly older population, and increasing rates of obesity and type 2 diabetes, the prevalence of VCI and HFpEF can only increase. Importantly, VCI and HFpEF often present together [9]. The brain and the heart are both sensitive to acute and chronic reductions in perfusion. Both have high-energy demands compared to total body mass and very limited energy storage capacity [10, 11]. Additionally, both neurons and cardiomyocytes lack the ability to replicate or regenerate in case of injury. Chronic HF patients have a significantly increased risk of white matter hyperintensity burden and cognitive impairment [12, 13]. On longitudinal follow-up, elderly patients with HF at baseline had the steepest decline in cognitive function and those that developed HF had lower baseline cognition [14]. Furthermore, cognitive function can be enhanced with improvement of cardiac function [15–17]. It is becoming increasingly recognized that VCI and HFpEF are different clinical manifestations of similar, if not the same, underlying pathological processes. Tini et al. [18] proposed that "HFpEF is a dementia of the heart."

One of the proposed common pathological processes is microvascular rarefaction. However, the knowledge about the role of rarefaction (i.e., the reduction in microvessel density) in VCI and HFpEF is still sparse [19]. Sporadic pathological studies in patients with cerebral white matter disease showed a decreased vascular density in brain tissue [20]. In a mouse model of CADASIL (cerebral autosomal dominant arteriopathy with subcortical ischaemic strokes and leukoencephalopathy), a mono-genetic type of cerebral microvascular disease in which VCI is an important clinical expression, a progressive reduction in capillary density has been reported [21]. Furthermore, there is pathological evidence of a reduction in the number of small arteries and capillaries in the brain of hypertensive diabetic and obese rodents [22, 23], and a decline in microvascular density in the hippocampus and cortex of these rodents correlated with cognitive function [24].

An autopsy study of HFpEF patients with non-cardiac death identified 27% reduction in microvessel density compared to controls [25]. Microvascular dysfunction is present in up to 75% of those with HFpEF, in the absence of significant coronary artery disease [26]. There are reduced coronary flow reserve and increased index of microvascular resistance [27] on invasive angiography that correlates with increased left ventricle (LV) end-diastolic pressure. Index of microvascular resistance increase may relate to microvascular rarefaction based upon animal experiments in which rarefaction was artificially induced [28].

Further evidence for the shared role of rarefaction in the pathogenesis VCI and HF comes from studies revealing that comorbidities, which are strongly associated with the development of both VCI and HF, in turn have

been shown to be associated with rarefaction. Obesity is associated with rarefaction in animal models and mid-life obesity is a risk factor for developing dementia and HFpEF [29–32]. Hypertension, especially in middle age, is a proven risk factor for both VCI and HFpEF [33, 34]. Hypertensives, and those with a family history of hypertension that yet have to develop hypertension, exhibit skin rarefaction [35]. The presence of DM doubles the risk for VCI and is associated with poorer outcomes in HFpEF [36, 37]. Retinal microvascular rarefaction has been demonstrated in DM [38], and rarefaction has been shown to cause insulin resistance preceding DM [39, 40]. The presence of rarefaction in a preclinical setting highlights it not only as an important diagnostic marker of future disease but also as a potential causative mechanism in the origin of VCI and HFpEF.

Non-histological direct quantification of microvessels in the heart and brain is not feasible with current techniques, but it is likely that rarefaction parallels microvascular dysfunction that can be quantified in vivo via various techniques. Brain magnetic resonance imaging (MRI) can assess multiple functional microvascular characteristics such as microvascular perfusion, vessel wall permeability, and cerebrovascular reserve capacity and can also sense various sizes of small blood vessels [41]. Cardiac MRI (CMR) can indirectly assess rarefaction due to its ability to quantify myocardial blood flow [42], and quantification of fibrosis which is associated with microvascular dysfunction [25, 43]. Peripheral microvascular density can be directly assessed sublingually, using the Glycocheck video microscope system [44], and in the retina, using optical computed tomography angiography (OCTa) [45]. Blood biomarkers provide another indirect method of assessing microvascular rarefaction in the heart-brain axis, via the analysis of endothelium-derived extracellular vesicles that are membrane-enclosed vesicles released from endothelial cells into the extracellular space [46] in response to cell activation or injury.

Our knowledge regarding underlying pathological and physiological processes in VCI and HFpEF remains incomplete, partly due to the inability of directly assessing microvascular rarefaction non-histologically in the heart and the brain. A better understanding of the relevance of microvascular rarefaction and methods to accurately measure microvascular density in the brain and the heart is needed to develop markers for early diagnosis and to identify targets for disease-modifying treatments. Here, we describe the study protocol of a multidisciplinary clinical study, designed to investigate microvascular rarefaction as a potentially important, but understudied pathophysiological mechanism in VCI and HFpEF. The clinical CRUCIAL (miCRovascular rarefaction in vascUlar Cognitive Impairment and heArt faiLure) studies are part of the larger CRUCIAL consortium [47], covering preclinical, experimental, and clinical research.

Study Aims

The CRUCIAL consortium endeavours to investigate the relevance of microvascular rarefaction in VCI and HFpEF. Furthermore, we will evaluate early biomarkers of rarefaction that can be used for screening, diagnosis, and therapeutic monitoring in VCI and HFpEF. We will investigate the use of novel non-invasive measures in the form of sublingual videomicrosopy (GlycoCheck), OCTa, and circulating endothelial extracellular vesicles alongside advanced brain MRI and CMR (Fig. 1).

Our aims include: (1) to investigate if microvascular perfusion and function measurements by MRI can identify microvascular rarefaction in the brain and the heart; (2) to relate rarefaction to cognitive and cardiac function in patients with VCI and HFpEF; (3) to determine the relationship between cerebral and cardiac microvascular rarefaction; (4) to investigate whether microvascular alterations are disease specific and not just an ageing phenomena; and (5) to investigate non-invasive measures that can be used as surrogate biomarkers for cerebral and/or cardiac rarefaction (sublingual capillary health, retinal angiographs, and biomarkers isolated from circulating endothelial extracellular vesicles).

Protocol Description

Study Design and Sites

CRUCIAL will compile four prospective observational cohorts covering a spectrum of deeply phenotyped participants with *VCI* and *HFpEF*, collected independently at four sites: VCI (Maastricht University Medical Centre, Maastricht, the Netherlands), HFpEF (Clínica Universidad de Navarra, Pamplona, Spain), HFpEF with severe symptomatic aortic stenosis (AS) (St Bartholomew's Hospital, London, UK), and aging controls with mixed comorbidities (AGE) (Medical Research Council Unit for Lifelong Health and Ageing, University College London, United Kingdom). VCI, HFpEF, and AGE participants are assessed cross-sectionally, and AS participants will additionally have a follow-up visit 6 months post-operatively.

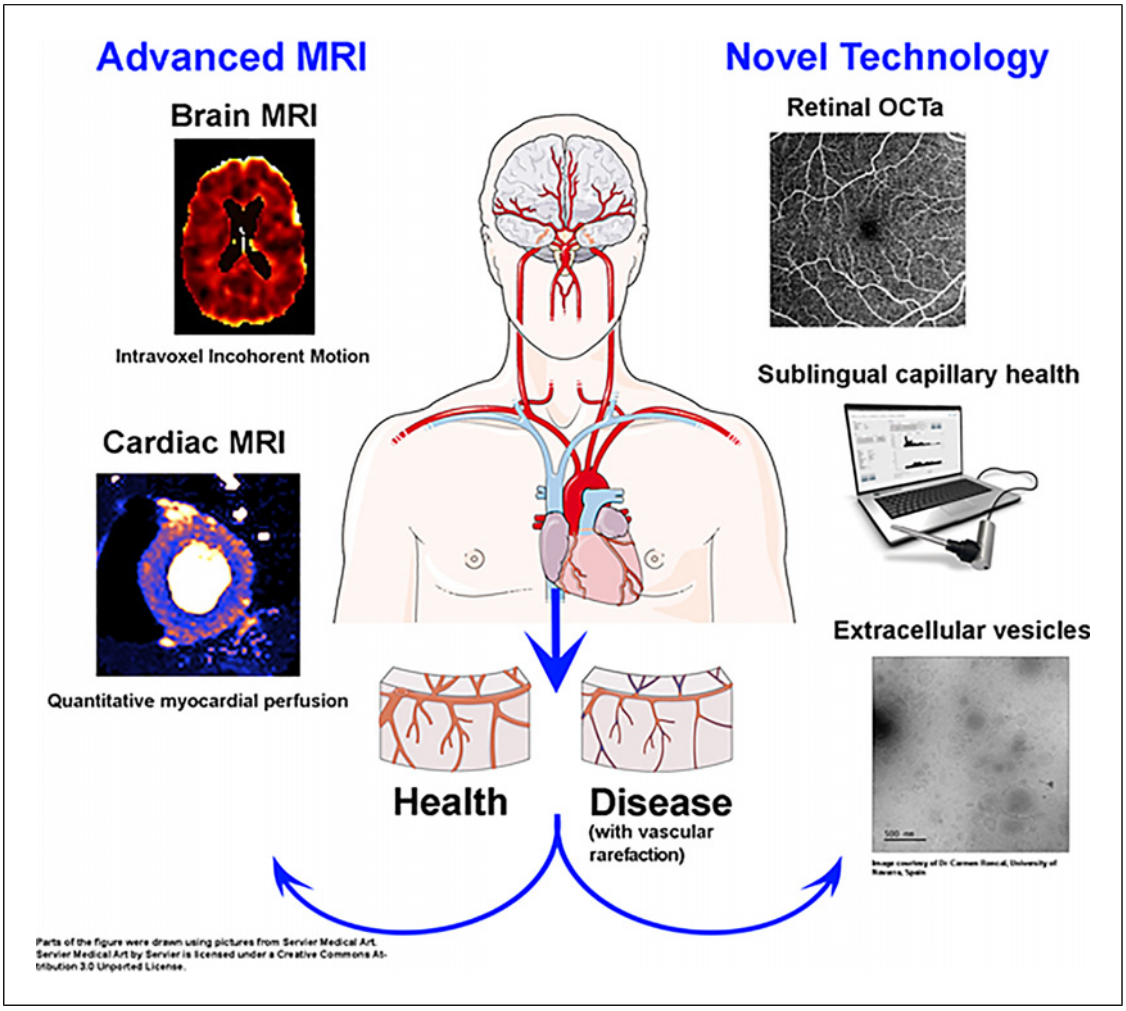

Fig. 1. CRUCIAL study overview.

Data are stored in accordance with local data protection policies and all sites adhere to FAIR principles (data to be findable, accessible, interoperable, and reusable). All participants provide written informed consent to take part. The CRUCIAL research protocols have been approved by research and medical ethics committees of the Maastricht University Medical Center in the Netherlands (Ref: NL72696.068.20), The Universidad de Navarra in Spain (Ref: 2019.210), and England and Scotland (Ref: IRAS 254776 & 275360).

Participants

In total, 395 participants are planned to be recruited, comprising 75 VCI, 60 HFpEF, 60 AS, and 200 AGE. Table 1 outlines in- and exclusion criteria.

The VCI cohort is recruited from the outpatient clinic neurology and memory services of Maastricht University Medical Center and nearby regional hospitals, existing stroke registries, and from previous studies. All patients have clinical and radiological evidence of small vessel disease, have VCI defined as an objective cognitive deficit, or are at risk for developing cognitive impairment (Table 1). The HFpEF cohort is recruited from the Clínica Universidad de Navarra in Pamplona, Spain. It includes patients with preserved LVEF and no antecedents of severe coronary artery disease. It consists of patients at risk of HFpEF: asymptomatic patients with known risk factors (e.g., hypertension, DM, metabolic syndrome, chronic kidney disease) and cardiac remodelling (LVH and or LA dilatation and/or diastolic dysfunction; stage B of HF [48]); as well as patients with a previous clinical diagnosis of HFpEF (stage C of HF [48]), showing signs and symptoms of HF, cardiac remodelling, diastolic dysfunction, and elevated natriuretic peptides. The AS cohort is recruited from St Bartholomew's Hospital in London. All have severe symptomatic AS undergoing

Table 1. In- and exclusion criteria

Inclusion criteria	Exclusion criteria
VCI	
(At risk for) VCI due to cSVD defined as (1) cognitive complaints and demonstration of a cognitive deficit (MoCa <26 or impairment in at least 1 cognitive domain in neuropsychological assessment) Or (1) clinically manifest cSVD by an MRI confirmed lacunar stroke, at least 3 months after stroke to avoid acute stroke effects AND (2) imaging evidence of small vessel disease (extensive leukoaraiosis on CT or [early] confluent WMH on MRI [Fazekas score ≥2] or multiple punctate WMH on MRI [Fazekas 1] in combination with lacunes or microbleeds)	Other major neurological or psychiatric conditions affecting the brain and interfering with the study design (among which multiple sclerosis, Parkinson's disease, alcohol/drug abuse, major cortical stroke, major neurotrauma, brain tumours)
Clinical dementia rating scale ≤1	Contraindications* to MRI, gadolinium contrast agent, or adenosine administration†
Written informed consent	Unwillingness or inability to give written consent or mentally incompetent to give informed consent
HFpEF	
Patients with known cardiovascular risk factors (hypertension, diabetes mellitus, metabolic syndrome, chronic kidney disease) and preserved LVEF and: (1) Stage B HF: cardiac remodelling (left ventricular hypertrophy +/− left atrial dilatation +/− diastolic dysfunction) but asymptomatic (2) Stage C HF: Cardiac remodelling + elevated NT-proBNP + signs and symptoms of HF	Contraindications* to MRI, gadolinium contrast agent, or adenosine administration
Written informed consent	Diagnosis of cardiomyopathy (dilated, hypertrophic, hypertrophic cardiomyopathy, cardiac amyloidosis) History of known coronary artery disease Unwillingness or inability to give written consent
Heart failure with preserved ejection fraction with severe AS	
Severe aortic stenosis due to undergo surgical aortic valve replacement	Greater than 50% coronary stenosis on coronary angiography or CT
Written informed consent	Greater than moderate additional valvular lesion preoperatively Diagnosis of cardiomyopathy (dilated, hypertrophic, cardiac amyloidosis) Contraindications* to MRI, gadolinium contrast agent, or adenosine administration Unwillingness or inability to give written consent
Ageing controls with mixed comorbidities (AGE)	
Aging controls with comorbidities willing to participate	Contraindications* to MRI, gadolinium contrast agent, or adenosine administration
Written informed consent	Unwillingness or inability to give written consent

* Contraindication to MRI include pacemaker, metallic foreign body, claustrophobia, pregnancy, neurostimulator, and other kinds of implanted devices or insulin pump. Contraindications to gadolinium contrast agent include allergy or severe renal impairment (eGFR < 30 mL/min). Contraindications to adenosine administration in CMR include asthma and/or COPD, slow heart rhythm (<50 beats per minute), irregular heart rhythm (i.e., atrial fibrillation), AV block II-III, sick sinus syndrome, prolonged QT interval, and systolic blood pressure <90 mm Hg. † VCI patients with a contraindication to adenosine will be included in the study, but will not undergo CMR. VCI, vascular cognitive impairment; cSVD, cerebral small vessel disease; MoCa, Montreal Cognitive Assessment; CT, computed tomography; WMH, white matter hyperintensities; MRI, magnetic resonance imaging; HFpEF, heart failure with preserved ejection fraction; LVEF, left ventricular ejection fraction; HF, heart failure; NT-proBNP, N-terminal fragment of probrain natriuretic peptide.

Table 2. List of procedures for participants

	VCI	HFpEF	AS	AGE
Clinical data 15 min	X	X	X	X
Echocardiography 20 min	X	X	X	X[a]
ECG 5 min	X	X	X	X
Neuropsychological assessment 30 min (short), 45 min (extensive)	Extensive protocol	Short protocol	Short protocol	X[a]
Brain MRI scan 30 min (short), 90 min (extensive)	Extensive protocol	Short protocol	Short protocol[b]	Short protocol
CMR scan 35 min (short), 45 min (extensive)	Short protocol	Extensive protocol	Extensive protocol[b]	Extensive protocol
Blood sample (5 min)	X	X	X[b]	X
Glycocheck (10 min)	X	X	X	X
OCT angiography (10 min)	X	X	X	X
Cardiac biopsy			X	

VCI, vascular cognitive impairment; HFpEF, heart failure with preserved ejection fraction; ECG, electrocardiogram; MRI, magnetic resonance imaging; OCT, optical coherence tomography. [a]Relevant data extracted from UK National Survey of Health and Development (NSHD) cohort database if available from previous sweeps. [b]Follow-up investigation at 6-month follow-up in all (CMR scan and blood sample) or part (brain MRI) of the AS cohort.

open heart surgical aortic valve replacement. The myocardial response to severe AS and HFpEF (i.e., in the absence of significant aortic valve disease) is similar, making it an appropriate model for HFpEF. Progressive valvular narrowing leads to LV pressure overload, LV hypertrophy, rarefaction, and fibrosis that can result in diastolic dysfunction [49–51]. Diastolic dysfunction is detectable in up to 60% of patients with severe AS [52]. This cohort provides the opportunity to correlate the non-invasive measurements with histology of myocardial tissue obtained during surgery. This cohort will have repeat assessment at 6 months to assess the dynamic nature of rarefaction in response to relief of the insult. The AGE cohort is recruited from the UK National Survey of Health and Development (NSHD) study in collaboration with the MyoFit46 investigators [53]. NSHD is the longest running birth cohort study in the UK, comprising a sample of all singleton births during 1 week in 1946 [54]. Greater than 50% of the original cohort remain involved and have lifelong cardiovascular and neurological phenotyping [53]. We will keep an anonymous record (age, sex) of eligible participants who were approached but declined to participate.

Assessments

In all cohorts, both heart and brain will be investigated, but the focus and extent differ according to the prevailing disease. An overview of assessments is presented in Table 2. Assessments in the VCI cohort will be spread out over two visits, whereas in HFpEF, AS, and AGE, all assessments will be performed on the same day.

Clinical Data

In a standardized case record form, data on demographics (age, sex, ethnicity), medical history (cardiovascular disorders, risk factors, and cognitive impairment), medication use, anthropomorphic measurements (weight, height, peripheral and central blood pressure, pulse wave velocity), and lifestyle (smoking behaviour, alcohol consumption) are documented. All participants will undergo standard 12-lead electrocardiogram.

Echocardiography

2D echocardiography will be performed by trained physiologists or doctors in accordance with British Society of Echocardiography guidelines [9]. Standard views will be used to assess: chamber and valve structure and function, Doppler measurements for diastology, and LV global longitudinal strain. Concurrent cardiovascular disease will be determined (e.g., significant valvular disease, pericardial disease).

Neuropsychological Assessment

Cognitive status will be assessed by a standardized battery of cognitive tests designed to cover global cognitive function and three major cognitive domains including memory, information processing speed, and executive functioning. An overview of all test instruments and their cognitive domains is presented in Table 3 [10–15]. The neuropsychological tests are administered according to standardized test protocols by trained researchers. Level and years of education of the

Table 3. Neuropsychological measures and instruments

Cognitive domain	Instrument	VCI	HFpEF, AS
Global cognition	Mini-Mental State Examination (MMSE) [47]	x	x
Episodic memory	15 – Word Verbal Learning Test [48]	x	x
Information processing speed	Trail making test (TMT; parts A and B) [49]	x	x
	Digit Symbol Substitution Test (DSST) [50]	x	
	Stroop Colour Word Test (SCWT) [51]	x	x
Executive functioning	Verbal fluency test (60 s; animals, 3 letters) [52]	x	
	TMT [49]	x	x
	SCWT [51]	x	x

participants, and possible problems interfering with testing (e.g., vision and hearing problems) will be recorded. For each cognitive test in each participant, z-scores will be calculated (the difference between the individual raw score and the sample mean divided by the sample SD). Then for each participant, compound domain scores on memory, executive function, and processing speed will be calculated by averaging the z-scores of the tests within each domain, and an overall cognition compound score will be calculated taking the average of the three domain compound scores.

Brain Magnetic Resonance Imaging

The detailed scan protocols are described in Table 4 [16, 17]. The MRI scans will be performed on a 3.0 T scanner (Philips Ingenia CX [18] for VCI, Siemens Magnetom Skyra [54] for HFpEF, and Siemens Magnetom Prisma [54] for AS and AGE). The total protocol takes 90 min (with contrast agent) for VCI participants and about 30 min (non-contrast) for the HFpEF, AS, and AGE participants. The MRI protocols consist of a range of structural and perfusion imaging sequences. An example of ASL and IVIM MRI maps is provided in Figures 2, 3, respectively. MRI scans will be reviewed by experienced radiologists to identify possible incidental findings.

Cardiac Magnetic Resonance Imaging

CMR will be performed using an aligned protocol outlined in Table 5 [57]. Depending on clinical site, CMR will be undertaken on 1.5 T Siemens Magnetom Aera [54] (HFpEF, AS), 3 T Philips Ingenia CX [18] (VCI), or 3 T Siemens Magnetom Prisma (AGE). CMR protocol is abridged (35 min) for the VCI cohort, and remaining cohorts have an extended (45 min) protocol. Adenosine stress perfusion imaging will be undertaken as previously described [57] with participants abstaining from caffeine 24 h and dual cannulas for separate administration of adenosine and gadolinium contrast. Peak stress and rest blood pressure and heart rate are recorded. An example of CMR stress and rest perfusion maps is presented in Figure 4. CMRs will be reviewed by cardiologists or radiologists to identify possible incidental findings.

Myocardial Biopsy

Myocardial biopsies will be taken from the interventricular septum under direct vision as previously described [58], during aortic valve surgery in the AS cohort (Trucut or scalpel biopsy on surgeon preference). Half of the biopsy material is immediately frozen in liquid nitrogen and the other half is fixed in buffered formalin (4%), where after it is paraffin-embedded. We will perform a histopathological assessment of cardiomyocytes' hypertrophy and fibrosis, and we will perform an immunohistochemistry against von Willebrand factor, an endothelial cell marker, to identify capillary blood vessels and vascular density will be calculated. The myocardial biopsy will allow us to relate microvascular perfusion measured with MRI to microvascular density in human tissue samples.

Retinal Optical Computed Tomography Angiography

Retinal OCT and OCTa will be used to assess the retinal microvasculature non-invasively. Images will be acquired in both eyes. OCT will be taken with both macula and optic nerve fixation: OCTa with macula fixation only. Automated software processes the image giving segmentation of individual retinal layers at different depths and an analysis of optic nerve and retina vascular morphology (Fig. 5) [59]. Images will be reviewed by ophthalmologists to identify possible incidental findings.

Sublingual Microvasculature

The microvasculature will be assessed non-invasively via the GlycoCheck system (Fig. 6 and online suppl. video

Table 4. Detailed MRI protocol for brain imaging

MRI technique	Purpose	Parameter	VCI	HFpEF, AS, AGE	Details
T1 3D fast gradient echo	Anatomy Segmentation and pathology	Total brain volume, lacunes, perivascular spaces, total cSVD burden score [55]	x	x	MP2RAGE, 1 mm cubic voxel size, two inversion times to map T1 value
Multi-slice T2 + proton density					Turbo spin echo
FLAIR 3D	White matter hyperintensities (WMH)	WMH volume, Fazekas score [56], total SVD burden score	x	x	
SWI	Microbleeds	Microbleeds, total cSVD burden score	x	x	T2*-FFE
IVIM	Microstructural integrity and microvascular perfusion (white and grey matter)	Parenchymal diffusion (D), microvascular perfusion volume fraction (f), and microvascular diffusivity (D*)	x	x	Diffusion imaging with the range of b values
ASL	Grey matter perfusion	Grey matter perfusion (CBF)	x	x	PCASL Visualization of carotid arteries required for the planning of the labelling plane
T1-weighted DCE and 2 post-contrast M2RAGEs	Blood-brain barrier leakage	BBB leakage rate + blood (plasma) fractional volume	x		Second post-contrast MP2RAGE >20 min after contrast agent administration, 0.1 mmol/kg gadobutrol bolus
Hybrid T2/T2* DSC	Macro- and microvascular perfusion (white and grey matter)	Cerebral blood volume (CBV), cerebral blood flow (CBF), mean transit time (MTT) Vessel size index	x		0.1 mmol/kg gadobutrol bolus

MRI, magnetic resonance imaging; VCI, vascular cognitive impairment; HFpEF, heart failure with preserved ejection fraction; MP2RAGE, magnetization prepared 2 rapid gradient echo; FLAIR, fluid-attenuated inversion recovery; SWI, susceptibility-weighted imaging; IVIM, intravoxel incoherent motion; ASL, arterial spin labelling; pCASL, pseudo-continuous arterial spin labelling; DCE, dynamic contrast enhanced; DSC, dynamic susceptibility contrast.

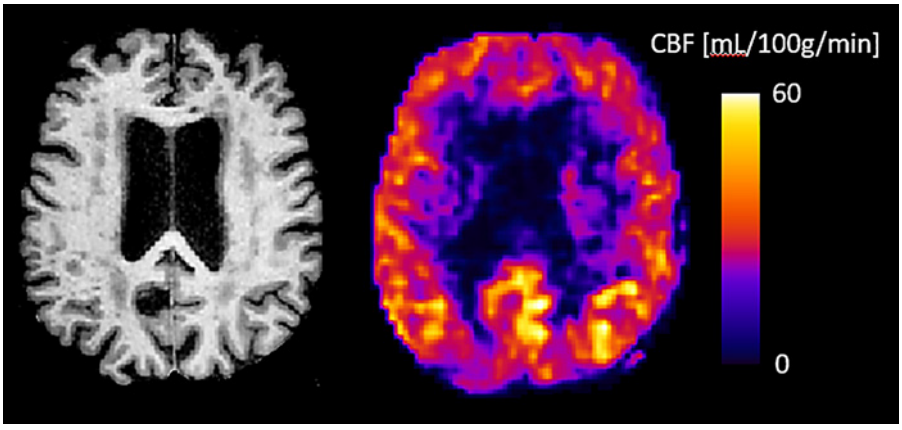

Fig. 2. Example of T1-weighted MR image of the brain and ASL (arterial spin labelling) map displaying cerebral blood flow (CBF) for a vascular cognitive impairment patient.

1; for all online suppl. material, see www.karger.com/doi/10.1159/000529067) that uses a handheld portable videomicroscope placed under the tongue [44]. Acquisition is roughly 10 min for the participant and is painless. Automated software analyses data from vessels between 5 and 25 μm in size for calculation of microvascular density, red blood cell content, valid density, and perfused boundary layer.

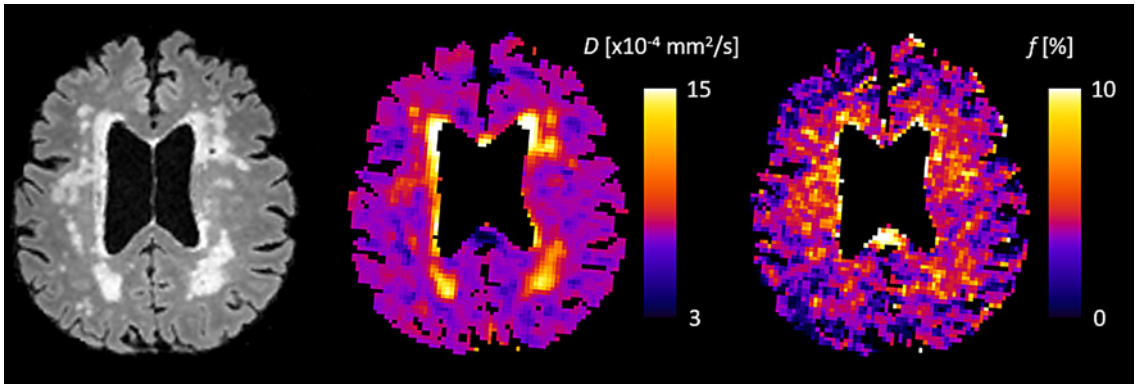

Fig. 3. Example of a FLAIR (fluid-attenuated inversion recovery) MR image of the brain displaying white matter hyperintensities and IVIM (intravoxel incoherent motion) maps of parenchymal diffusivity D and the perfusion fraction f for a vascular cognitive impairment patient.

Table 5. Detailed MRI protocol for cardiac imaging

CMR sequence	Purpose	Parameter	VCI	HFpEF, AS, AGE	Details
Localizers	Localizers, incidental findings		x	x	Single-shot multi-plane
Axial anatomical stack			x	x	White blood, black blood
Single shot			x	x	Single-shot multi-plane
Frequency scout				x	Fast low-resolution images at different RFs
Long-axis bSSFP	Anatomy, volumes, and function	LA & RA volumes and function, MAPSE, TAPSE, LV strain	x	x	Breath-held, balanced, steady-state-free precession cines
Native T1 mapping	Diffuse fibrosis	Native T1 value (ms)	x	x	MOLLI 5(3)3 T1 mapping
Post-contrast T1 mapping, extracellular volume (ECV)		ECV (%) and indexed ECV (mL/m^2)	x	x	MOLLI 4(1)3(1)2
Native T2 mapping	Oedema	T2 value (ms)	x	x	T2-prepared SSFP
Aortic valve flow mapping	Aortic valve velocities	AV-Vmax (cm/s)		x	Phase-contrast breath-held gradient echo
Adenosine stress perfusion	Micro- and macrovascular perfusion	MBF (mL/kg/min)	x	x	Automated inline quantitative perfusion mapping [57]
Short axis cine bSSFP	Ventricular volumes, function, mass	LV and RV indexed volumes and systolic function (EDV (mL), ESV (mL), ejection fraction (%), stroke volume (mL), LV mass index (g/m^2), LV maximal wall thickness (mm)	x	x	Breath-held, balanced, steady-state-free precession cines
Late gadolinium enhancement	Focal scar	LGE quantification	x	x	Phase-sensitive IR bright and dark blood

LA, left atrium; RA, right atrium; MAPSE, mitral annular plane systolic excursion; TAPSE, tricuspid plane systolic excursion; LV, left ventricle; ECV, extracellular volume; AV-Vmax, aortic valve maximal velocity; MBF, myocardial blood flow; EDV, end-diastolic volume; ESV, end-systolic volume; LGE, late gadolinium enhancement; RF, radiofrequency; MOLLI, modified Look-Locker inversion recovery; (b)SSFP, (balanced) steady-state-free precession; IR, inversion recover.

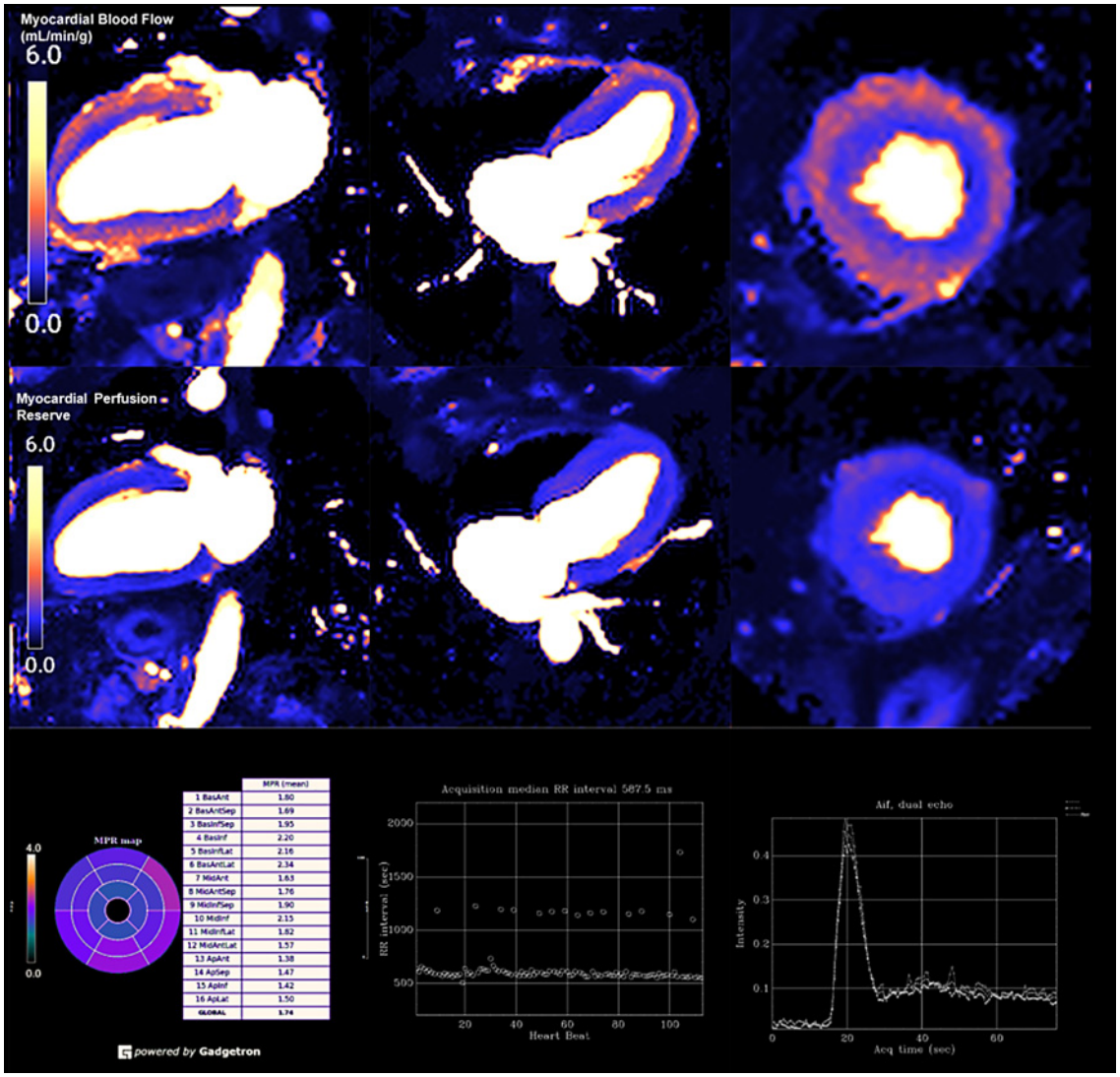

Fig. 4. Example of CMR stress (top) and rest (bottom) perfusion maps with myocardial blood flow mapping in a participant with severe aortic stenosis with quality assurance outcomes.

Blood Sampling

All participants will undergo fasting blood sampling for serum and platelet-poor plasma for biomarkers and extracellular vesicle analysis. Serum and platelet-poor plasma will be conventionally processed, all stored at −80°C, and analysed at CIMA Universad de Navarra, in Pamplona, Spain. Endothelium-derived extracellular vesicles will be counted by flow cytometry using endothelial-specific antibodies. Endothelium-derived extracellular vesicles will be isolated by immunobead capture and an ultra-low input RNA-Seq protocol will be applied. Circulating non-coding RNAs will be also evaluated.

We will also measure a panel of circulating biomarkers related to cardiac remodelling including N-terminal fragment of probrain natriuretic peptide; high-sensitivity troponin T to assess cardiomyocyte injury; biomarkers associated with fibrosis such as the pro-collagen type IC-terminal propeptide, the collagen type I C-terminal telopeptide, matrix metalloproteinase-1, and inflammation like galectin-3 or soluble ST-2; and biomarkers of endothelial dysfunction like adhesion molecules (e.g., VCAM-1, ICAM-1).

Sample Size Considerations

With a sample size of 60–75 participants in the clinical cohorts, anticipating a dropout (failed or incomplete

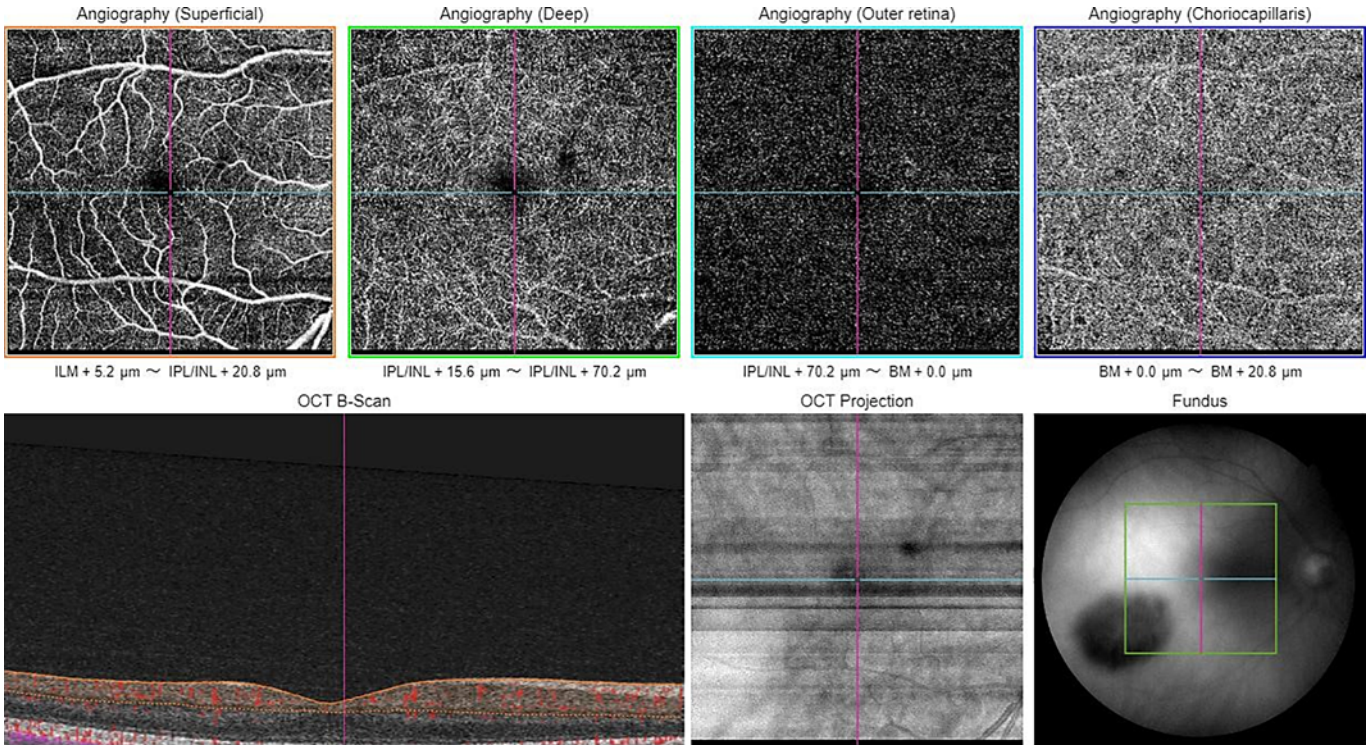

Fig. 5. OCTa images of the multiple layers of the retina in a participant with severe aortic stenosis.

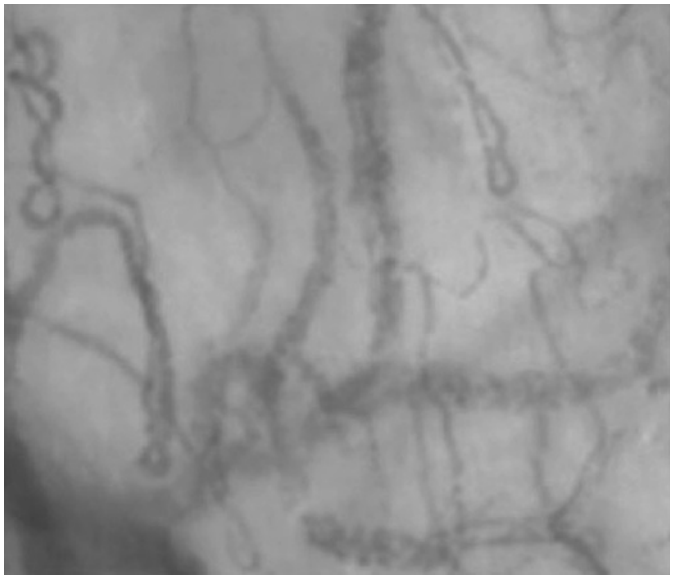

Fig. 6. Still image of the sublingual blood vessels obtained by the GlycoCheck system.

measurements) of 10–20%, we anticipate to detect a modest correlation (correlation coefficient of 0.3–0.4) between advanced MRI measures of microvascular function and density in brain and heart (i.e., cerebral blood flow, perfusion volume fraction, vessel size index, vessel density index, blood-brain barrier leakage volume and rate, and stress myocardial blood flow) and disease severity (i.e., cognitive function, extent of conventional structural MRI markers, and New York Heart Association functional class, and structural and functional markers of HFpEF), with power ≥80% (alpha = 0.05). Myocardial biopsies from 60 participants in the AS group will provide 84% power (alpha = 0.05) to detect a correlation r ≥ 0.4 between CMR stress MBF and histological parameters (capillary density).

Data Analysis

Brain MRI data will be analysed by the MUMC team. Structural images will automatically be segmented into grey matter and white matter using FreeSurfer software [60], whilst white matter hyperintensities and infarcts will be manually segmented. ASL images are processed using the FSL BASIL tool for cerebral blood flow quantification [61]. IVIM data will be analysed using the biexponential IVIM model with incorporation of inversion recovery for cerebral spinal fluid suppression [62]. CMR data will be analysed in Circle Cardiovascular

Imaging, CVI42 version 5.14 [63] by the UCL team using a standardised protocol derived from society guidelines [64, 65] and as described previously [55]. LV mass will include papillary muscles and trabeculations, and tissue mapping will have 10% step-back from manually drawn epi- and endocardial contours and be split into 16-segments as per American Heart Association model. LGE presence will be assessed qualitatively and semi-quantitively via previously described methods [66]. CMR cine images will be analysed by a clinically validated artificial intelligence system proven to be superior to clinical experts [67].

All recruited participants will be reported to reflect the demographics of the study population. All participants providing data relevant to the analysis of interest will be analysed (unless examination provided no data). All participants where data were inadequate for analysis and the reasons will be reported. Baseline data will be summarized by means of simple descriptive statistics. Associations between measures of microvascular density and outcome measures (cognition, conventional structural MRI markers of cerebral small vessel disease, and heart function) will be quantified using general linear models with appropriate link functions or if necessary zero inflated models. Adjustment for potential confounders (e.g., age, sex, hypertension, diabetes, prior cardiovascular disease, renal disease) will be performed based upon prior knowledge.

Current Status and Timeline

The first VCI participant was included in December 2020, the first AS and AGE participants were included in September 2021, and the first HFpEF participant was included in December 2021. Recruitment is anticipated to finalize in all cohorts by the end of 2024 inclusive of follow-up in the AS cohort, with first results expected in 2025. The COVID pandemic delayed setup and start of recruitment at all sites, ranging from 5 to 17 months, with further delay from slower than anticipated initial recruitment rates.

Discussion

For both HFpEF and VCI, early diagnosis of pathophysiological changes is essential because both the heart and the brain are organs that we currently cannot repair after they are damaged. Clinical diagnosis of both VCI and HFpEF is difficult. The diagnosis of HFpEF, as reflected in the current guidelines, relies on the onset of clinical symptoms together with surrogate makers of cardiac dysfunction (elevated natriuretic peptides and echocardiographic assessment of diastolic dysfunction). In VCI, diagnosis relies on macrostructural brain lesions determined by MRI (such as white matter hyperintensities, lacunes, and microbleeds) or CT (leukoaraiosis). However, these diagnoses are made at the stages that comorbidities already have an irreversible effect on the heart and the brain. If rarefaction is the first step, then measuring rarefaction will allow early diagnosis, which implies possibilities for prevention and risk stratification. Besides, a better knowledge of the relevance of microvascular rarefaction and its underlying mechanisms might provide new targets for prevention and disease progression. Targeting the common pathways of microvascular rarefaction, rather than neurons or cardiomyocytes directly, could open new doors to therapeutics that benefit both organ systems simultaneously.

The CRUCIAL project aims to investigate the role of rarefaction in HFpEF and VCI within 4 comprehensively phenotyped prospective cohorts. Our design will uniquely combine advanced MRI, new technologies, and histology to drive forward knowledge in the field. Although there is substantial evidence that VCI and HFpEF often present together and have common underlying pathophysiological processes, most previous studies focused on a single patient group or a single organ. In CRUCIAL, all participants undergo structural and perfusion imaging of both the brain and the heart, regardless of the prevailing disease. Combining the structural brain and cardiac and cognitive data with perfusion MRI techniques will shed new light on structural and dynamic features of the heart-brain axis. The comprehensive multimodal brain imaging protocol, including a combination of perfusion, diffusion, BBB permeability, and vessel size imaging, will provide a unique extensive dataset. We will adopt a challenging approach to combine these different functional brain MRI measures into a compound measure for cerebral microvascular function. Only a few previous studies have used multimodal cerebral microvascular function imaging [68–71]. The AGE cohort from NSHD will provide new insights into rarefaction within the context of lifelong aging exposures and outcomes. Myocardial biopsy from the AS cohort will allow us to make a direct correlation of microvascular density with myocardial perfusion by CMR and other non-invasive techniques. Additionally, since this cohort will have a follow-up, we can investigate whether microvascular function can respond in relief of the insult. Our utilization of novel non-invasive measures for faster, simpler, and cheaper assessment of rarefaction is more likely to be translated to widespread clinical practice with the expected benefit of reducing the vast costs associated with HFpEF and VCI. Though these technologies are being developed

as stand-alone technologies, a combination of tools might be more effective at identifying rarefaction than any measure alone, as comorbidities are systemic and microvascular rarefaction is likely to affect more than one organ. For this reason, testing all these technologies in one single subject is a real strength of the project. Finally, the preclinical studies within the CRUCIAL consortium will further increase our understanding of the mechanisms underlying microvascular rarefaction.

However, some limitations of the study protocol must be acknowledged. Many of techniques used in these studies measure perfusion, which can be regarded as measure for functional rarefaction, rather than structural rarefaction. Although we will correlate the CMR to myocardial biopsies in the AS cohort, we cannot validate brain MRI measures since pathological research in the brain is only possible post-mortem. For this, we will depend on the preclinical part of CRUCIAL [47] in which animal models will be used. Since the largely cross-sectional design of the study, we will not be able to claim about causality between microvascular rarefaction and disease severity, nor can we examine the evolution of rarefaction over time and determine whether further decrease in vessel density predicts further deterioration of cardiac function and cognitive decline.

In conclusion, in CRUCIAL, we investigate the relevance of microvascular rarefaction as an underlying pathophysiological process in both VCI and HFpEF. Moreover, we will validate advanced brain and CMR measures for microvascular function and density and develop novel non-invasive measures for detection of microvascular rarefaction using sublingual video-microscopy, OCTa, and circulating endothelial extracellular vesicles.

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Statement of Ethics

All participants provide written informed consent to take part. The CRUCIAL research protocols have been approved by research and medical ethics committees of the Maastricht University Medical Center in the Netherlands (Ref: NL72696.068.20), The Universidad de Navarra in Spain (Ref: 2019.210), and England and Scotland (Ref: IRAS 254776 & 275360).

Conflict of Interest Statement

F. Barkhof is in the steering committee or iDMC member for Biogen, Merck, Roche, EISAI, and Prothena, and consultant for Roche, Biogen, Merck, IXICO, Jansen, and Combinostics. He also has research agreements with Merck, Biogen, GE Healthcare, and Roche, and he is co-founder and stakeholder of Queen Square Analytics LTD. Other authors have no conflicts of interest to declare.

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Author Contributions

J.F.A. Jansen, G. Captur, A. Hughes, J. Staals, W.H. Backes, G. Bastarika, E.A.V. Jones, A. González, R.J. van Oostenbrugge, and T.A. Treibel contributed to the study conception. M. van Dinther, J. Bennett, G.D. Thornton, P.H.M Voorter, A. Ezponda Casajús, G. Captur, A. Hughes, Robert J. Holtackers, J.F.A. Jansen, J. Staals, W. Backes, G. Bastarika, A. González, R.J. van Oostenbrugge, and T.A. Treibel contributed to collection and analysis of data. The first draft of the manuscript was written by M. van Dinther, J. Bennett, and G.D. Thornton, and all authors reviewed and commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this article and its online supplementary materials. Further enquiries can be directed to the corresponding author.

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