PROTOCOL FOR A PROSPECTIVE EXPERIMENT

The impact of accelerated pacing and AV-delay regulation on the pulmonary capillary wedge pressure during exercise in patients with HFpEF

APAVE

Version number: v5.0 – **Date** 14/04/2026

UZ/KU Leuven ref. number: S70922

Sponsor:

UZ Leuven (University Hospital Leuven)

Herestraat 49, B-3000 Leuven

Sponsor-Investigator:

Professor Dr. Peter Sinnaeve

Confidentiality Statement

The information in this document is strictly confidential and is available for review to Investigators, potential Investigators and appropriate Ethics Committees, Institutional Review Boards or Competent Authorities. No disclosure should take place without written authorization from the Sponsor.

LIST OF PARTICIPATING SITES

(as applicable)

List of participating sites	Principal Investigator *(must be a 'WUG' profession)*
UZ Leuven	Prof. Dr. Peter Sinnaeve
Jessa Hospital (Hasselt)	Dr. Jan Verwerft

SIGNATURES

Title: The impact of accelerated pacing and AV-delay regulation on the pulmonary capillary wedge pressure during exercise in patients with HFpEF

Protocol: APAVE

The undersigned confirm that the above referenced protocol has been acknowledged and accepted, and agree to conduct the Study in compliance with the approved protocol, and will adhere to: the ICH guidelines, the most recent version of the Declaration of Helsinki, the Belgian law of May 7th 2004 regarding experiments on the human person, the EU General Data Protection Regulation 2016/679 (GDPR), the Belgian law of July 30th 2018 on the protection of natural persons with regard to the processing of personal data, the Belgian Law of August 22nd 2002 on participant rights, and any other regulatory requirements and Standard Operating Procedures (SOPs), as applicable.

The undersigned agree not to disclose the confidential information contained in this document for any purpose other than the evaluation or conduct of the Study, without prior written consent of the Sponsor.

The undersigned Sponsor-Investigator also commit to making the findings of the Study publicly available through publication and/or other dissemination tools, in accordance with this protocol and applicable regulations, without any unnecessary delay and to provide an honest, accurate and transparent account of the Study; and to explain any discrepancies or deviations from the approved Study protocol.

Sponsor-Investigator:

.....	.....	.....
Name & Title	Signature	Date

Principal Investigator of participating site:

.....	.....	.....
Name & Title	Signature	Date

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Sponsor-Investigator:

.....	.....	.....
Name & Title	Signature	Date

Principal Investigator of participating site):

.....	.....	.....
Name & Title	Signature	Date

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
AESI	Adverse Event of Special Interest
APR	Annual Progress Report
ASAP	As soon as possible
ASR	Annual Safety Report
AR	Adverse Reaction
CA	Competent Authorities
CSR	Clinical Study Report
CTC	Clinical Trial Center
CME	Centrum Menselijke Erfelijkheid
DMP	Data Management Plan
DTA	Data Transfer Agreement
DPA	Data Processing Annex
EC	Ethics Committee
eCRF	electronic Case Report Form
ECG	Electrocardiogram
EU	European Union
EoS	End of Study
FPFV	First Participant First Visit
GCP	Good Clinical Practice (latest version of ICH E6)
GDPR	EU General Data Protection Regulation 2016/679
HBM	Human Body Material
IC(F)	Informed Consent Form
ICH	International Council on Harmonisation
ISF	Investigator Site File
IUD	Intra-uterine device
IMP	Investigational Medicinal Product
IMD	Investigational Medical Device
IVRS/IWRS	Interactive Voice/Web Reporting System
LAG	Laboratoriumgeneeskunde
LPLV	Last Participant Last Visit
MRI	Magnetic Resonance Imaging
PI	Principal Investigator (of participating research site)
PRO	Participant Reported Outcome
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
TMF	Study Master File
VIB	Vlaams Instituut voor Biotechnologie

WUG	Wet uitoefening gezondheidszorgberoepen
PCWP	Pulmonary Capillary Wedge Pressure
LVFP	Left ventricular filling pressures
CO	Cardiac output
SV	Stroke volume
VO2	Oxygen uptake
HFpEF	Heart failure with preserved ejection fraction
EDPVR	End-diastolic pressure-volume relationship
CI	Chronotropic incompetence
iLVESV	Indexed left ventricular end-systolic volume
PVC	Premature ventricular contractions
iCPET	Invasive cardiopulmonary exercise test
LAVI	Left atrial volume index
LVMI	Left ventricular mass index
RWT	Relative wall thickness
GLS	Global longitudinal strain
LV	Left ventricle
TR	Tricuspid regurgitation
SaO2	Arterial oxygen saturation
SvO2	Mixed venous oxygen saturation
RPM	Rotation per minute
APMHR	Age predicted maximal heart rate
NYHA classification	New York Heart Association classification
RER	Respiratory Exchange Ratio
AV-delay	Atrioventricular delay
SWV	Shear wave velocity
W	Watts

FUNDING & SUPPORT

Funder or service provider	Type of Support (financial or non-financial)
Prof. Dr. Peter Sinnaeve	Financial

Reasonable travel expenses (such as public transport, parking fees or mileage reimbursements) incurred by study participants and directly related to participation in the study, will be reimbursed by the Sponsor. Moreover, compensation for time investment will be provided (€13 per hour) and participants will receive an additional compensation for discomfort caused by the invasive measurements (€100).

A travel reimbursement payment log will be kept by the Investigator Site staff (as applicable) and made available for monitoring and verification purposes.

ROLES & RESPONSIBILITIES

The Principal Investigator (PI) is responsible for the conduct of the study at his/her participating research site, and for protecting the rights, safety and well-being of the study participants. As such the PI must ensure adequate supervision of the study conduct at the participating site. If any tasks are delegated, the PI will maintain a log of appropriately qualified persons to whom he/she has delegated specified study-related duties. The PI will ensure that adequate training is provided and documented for all study staff, prior to conducting assigned study-related activities.

It is the Sponsor-Investigator's responsibility to supervise the general conduct of the study (e.g. study progress, communication, protocol training and support of the participating research sites, annual reporting to the Ethics Committee (EC), end of study notification(s) and results reporting, etc.). The UZ/KU Leuven Sponsor-Investigator fulfills mostly both Investigator **and** Sponsor responsibilities, as outlined in latest version of the International Council on Harmonisation – Good Clinical Practice (ICH-GCP) E6 and applicable regulations.

PI and Sponsor-Investigator shall each be referred to as «Investigator(s)».

STUDY SYNOPSIS

Title of clinical study («study»)	The impact of accelerated pacing and AV-delay regulation on the pulmonary capillary wedge pressure during exercise in patients with HFpEF
Protocol number / acronym	APAVE
Sponsor name	UZ Leuven (University Hospital Leuven)
Sponsor-Investigator	Professor Dr. Peter Sinnaeve
Contact address:	Herestraat 49, 3000 Leuven
Contact email:	Peter.sinnaeve@uzleuven.be
Contact phone:	016342302
Study nbr in public database	NCT07270536
Medical condition or disease under investigation	Heart failure with preserved ejection fraction (HFpEF)
Study rationale	HFpEF, characterized by elevated left filling pressures and exercise intolerance, is an increasingly common clinical syndrome. Exercise intolerance is the primary symptom and significantly impacts quality of life. An elevated pulmonary capillary wedge pressure (PCWP), a marker of increased left atrial pressure (LAP), is associated with reduced exercise capacity, likely due to pressure transmission to the lungs, and increased mortality. Research has shown that increasing the heart rate (HR) at rest can reduce LAP in patients with HFpEF, potentially enhanced by a shortened AV-delay. However, patients mainly experience symptoms during low-intensity exercise, where the effects of accelerated pacing remain unknown.
Primary objective	To assess the impact of accelerated pacing with AV-regulation on the PCWP during exercise in patients with HFpEF, particularly at lower intensities of exertion, to offer insights into pacing-based interventions to ultimately enhance exercise tolerance and quality of life.
Secondary objective(s)	To assess the impact of accelerated pacing with AV-regulation on cardiac output, stroke volume, peripheral oxygen extraction and shear wave velocity.
Endpoints	The primary endpoint is the change in PCWP for each pacing frequency compared to the baseline HR, assessed at rest and during exercise (cycle ergometry at 25 W). The baseline HR is defined as the intrinsic HR or baseline atrial pacing rate, as applicable. Secondary endpoints include changes in cardiac output, stroke volume, and peripheral oxygen extraction from the baseline HR to the pacing frequency associated with the greatest reduction in PCWP, both at rest and during exercise. Additionally, changes in shear wave velocity at each pacing frequency relative to the baseline HR will be assessed.
Sample Size	20

Date of anticipated First Participant First Visit (FPFV)	03/03/2026
Date of anticipated Last Participant Last Visit (LPLV)	02/03/2027
Expected study duration for a single participant	3 weeks

STUDY FLOWCHART

Schedule of events – Study specific procedures & assessments

UZ Leuven

Procedures/ Assessment	**Screening**	**Screening visit 2 (only when necessary)**	**Study visit**
Visits / Contacts	**Visit 1**	**Visit 2**	**Visit 3**
Timing (weeks)		**+ 1 week**	**+ 3 weeks**
Visit Window (days)	**1 day**	**From the day of screening up to 3 months after screening visit 1**	**Up to 4 months after screening visit 1**
Informed consent	X [1]		
Inclusion / Exclusion criteria	X		
Demographics	X		
Medical / Surgical history	X		
Physical examination	X		
Weight / Height	X		X
Vital Signs	X		X
12-lead ECG	X		X
Haematology sampling	X		
Coagulation sampling	X		
Chemistry sampling	X		
(Serious) Adverse event (S)(AE) assessment			X
Invasive CPET (2)			X
Transthoracic echocardiography	X		X
Exercise stress echocardiography		(X)[3]	
Arterial blood sampling			X
Venous blood sampling			X

Footnotes

Study-specific procedures are labelled in blue; non–study-specific procedures are labelled in green. An ECG, transthoracic echocardiography (TTE), and haematology, coagulation, and chemistry sampling are expected to be performed as part of standard of care. If these assessments have not been performed, or if no recent results are available (within ≤3 months), they will be conducted and considered study-specific procedures.

(1) Informed consent must be obtained prior to performing any other study-related procedures

(2) Invasive CPET on a semi supine cycle ergometer

(3) This test will be conducted exclusively in patients with an HFA-PEFF score 2-4 when calculated without functional testing

Jessa Hospital

Procedures/ Assessment	**Screening**	**Screening visit 2**	**Screening visit 3 (only when necessary)**	**Study visit**
Visits / Contacts	Visit 1	Visit 2	Visit 3	Visit 4
Timing (weeks)		**+ 1 week**	**+ 2 weeks**	**+ 3 weeks**
Visit Window (days)	1 day	**From the day of screening visit 1 up to 3 months after**	**From the day of screening visit 2 up to 1 month after**	**Up to 4 months after screening visit 2**
Informed consent		X [1]		X [1] (when no visit 2 necessary)
Inclusion / Exclusion criteria	X	X		
Demographics	X	X		
Medical / Surgical history	X	X		
Physical examination	X	X		

Weight / Height	X	X		X
Vital Signs	X	X		X
12-lead ECG	X			X
Haematology sampling	X	(X)		
Coagulation sampling	X	(X)		
Chemistry sampling	X	(X)		
(Serious) Adverse event (S)(AE) assessment				X
Invasive CPET (2)				X
Transthoracic echocardiography	X	(X)		X
Exercise stress echocardiography[3]			(X)	
Arterial blood sampling				X
Venous blood sampling				X

Footnotes

Study-specific procedures are labelled in blue; non–study-specific procedures are labelled in green. An ECG, transthoracic echocardiography (TTE), and haematology, coagulation, and chemistry sampling are expected to be performed as part of standard of care. If these assessments have not been performed, or if no recent results are available (within ≤3 months), they will be conducted and considered study-specific procedures in screening visit 2. The informed consent form (ICF) will be signed either at the start of visit 2 or at the start of the study visit, depending on whether a second screening visit is required.

(1) Informed consent must be obtained prior to performing any other study-related procedures

(2) Invasive CPET on a semi supine cycle ergometer

(3) This test will be conducted exclusively in patients with an HFA-PEFF score 2-4 when calculated without functional testing

1 Background, rationale & risk assessment

The role of filling pressures and heart rate in exercise intolerance among patients with HFpEF

Heart failure with preserved ejection fraction (HFpEF), defined by a left ventricular ejection fraction ≥50% along with evidence of elevated left ventricular filling pressure (LVFP) either at rest or during stress, is a prevalent and increasingly recognized clinical syndrome, affecting 1–3% of the global population. HFpEF accounts for approximately 50% of all heart failure cases, with its prevalence rising due to aging populations and increasing comorbidities such as hypertension, obesity, and type 2 diabetes. The disease burden is significant, leading to frequent hospitalizations, high healthcare costs, and substantial morbidity and mortality (1).

Exercise intolerance is a hallmark symptom of HFpEF and a major contributor to a reduced quality of life (2, 3). Elevated pulmonary capillary wedge pressure (PCWP), a surrogate for left atrial pressure, has been independently associated with impaired exercise capacity, regardless of traditional determinants of oxygen uptake (4). This association is likely driven by backward transmission of pressure leading to pulmonary congestion, impaired gas exchange, and ventilation–perfusion mismatch (5). Moreover, exertional increases in cardiac filling pressures have been linked to poorer survival outcomes (6).
Recent findings by Van Loon et al. revealed that accelerated pacing at rest reduces left atrial pressure (LAP) in patients with a high likelihood of HFpEF. Computer simulations suggest that this effect may be further enhanced when combined with AV-delay shortening (7). However, this combined approach has not yet been tested in actual patients and it remains unclear whether such pacing strategies confer similar hemodynamic benefits during exercise, precisely when symptoms tend to manifest. From a pathophysiological perspective, this relationship can be explained by the steep end-diastolic pressure-volume relationship (EDPVR) observed in HFpEF where increased ventricular stiffness leads to an exponential rise in left ventricular end-diastolic pressure (LVEDP) in response to even a slight reduction in heart rate (HR) and prolonged diastole (8). This mechanistic understanding provides a strong rationale to investigate whether similar hemodynamic dynamics occur during exercise, when exertional stress may further amplify these effects. It should be noted, however, that reductions in PCWP could also result from decreased cardiac output (CO), potentially due to a shortened diastolic filling period associated with accelerated pacing. This possibility has not yet been examined and therefore warrants further investigation, as a reduction in CO leading to forward failure would negate any potential benefits gained from a reduction in backward failure (9).

Conversely, during exertion, an impaired chronotropic response, known as chronotropic incompetence (CI), has been associated with reduced exercise capacity and provides independent and additive prognostic value relating it to higher mortality and an increased rate of heart failure events (10-14). However, it remains unclear whether this relationship is mediated through altered ventricular filling pressures. CI is highly prevalent in HFpEF with reported rates ranging from 34% to 63%, depending on the diagnostic criteria being used (12, 15).

Notably, a recent meta-analysis identified both CI and elevated left ventricular filling pressures as the most prevalent and pathophysiologically relevant hemodynamic disturbances in HFpEF, highlighting their therapeutic potential (16). Modulating HR by pacing strategies may simultaneously

improve chronotropic response and reduce PCWP, thereby targeting two key mechanisms of exercise intolerance.

Given that most daily activities involve low-to-moderate exertion, focusing on low-intensity exercise is particularly relevant, as functional improvements at these levels are likely to yield the greatest benefits in terms of patient autonomy and quality of life.

Approaches to modulate heart rate in patients with HFpEF

Discontinuation of negative chronotropic medication
One strategy to improve the chronotropic response in HFpEF is the withdrawal of negative chronotropic agents. Interestingly, it has been demonstrated that ivabradine further reduces exercise tolerance in HFpEF (17). Although a previous single-centre, retrospective study assessing peak HR and peak VO_2 in patients with HFpEF with and without β-blockers found no significant differences (18), the PRESERVE-HR study, a multicentred, randomized, investigator-blinded, crossover clinical trial, showed a significant increase in peak VO_2 following β-blocker discontinuation in patients with HFpEF and CI (19). Moreover, a lower indexed left ventricular end-systolic volume (iLVESV) may help identify those who experience greater short-term improvements in maximal functional capacity after β-blocker withdrawal (20). Currently, a meta-analysis on the effects of β-blocker withdrawal in patients with HFpEF is underway (21).

Rate-adapted and accelerated pacing
Pacing represents another strategy to modulate HR in patients with HFpEF. This approach is particularly feasible, as approximately 23% of these patients already have a pacemaker in place (22) . Additionally, atrial fibrillation, present in 40% to 60% of patients with HFpEF, is frequently managed with rate-controlling medications, potentially amplifying the therapeutic impact of pacing interventions in this subgroup (22).

The myPACE trial demonstrated that personalized accelerated pacing at rest, with a median lower rate of 75 bpm compared to the standard setting of 60 bpm, led to improvements in quality of life, NT-proBNP levels, physical activity, and device-detected atrial fibrillation (23). In contrast, the RAPID-HF study investigating pacemaker implantation with rate-responsive pacing in HFpEF patients with CI found no significant improvement in VO_2peak and VO_2 at VAT (24). This lack of effect may be attributed to several factors, including the small sample size (n = 29), the absence of accelerated pacing at rest, and a suboptimal chronotropic response during exercise. Specifically, excessive HR increases may compromise CO in HFpEF due to their impaired diastolic filling and a blunted pressure–volume relationship (25-27). Conversely, an insufficient HR rise may fail to adequately enhance chronotropic function or reduce PCWP, thereby limiting hemodynamic benefit. To address these uncertainties, we aim to investigate hemodynamic responses across multiple pacing frequencies. Moreover, this study has focused on high-intensity exercise, evaluating outcomes beyond the anaerobic threshold. In contrast, our study targets low-intensity exertion.

Shear wave velocity as a marker of ventricular stiffness in HFpEF

An additional parameter we will evaluate in this study is shear wave velocity (SWV). This imaging technique provides insights into myocardial stiffness by assessing the propagation speed of naturally occurring myocardial shear waves following mitral valve closure. A study by Salerno et al. demonstrated that higher pacing frequencies are associated with increased SWV, indicative of

elevated ventricular stiffness (28). However, these findings were observed in a population without HFpEF. In patients with HFpEF, who are characterized by increased ventricular stiffness and a steep EDPVR, the effects of pacing on ventricular stiffness and SWV is unknown. These insights may aid in the pathophysiological understanding of pacing strategies designed to improve diastolic function and exercise tolerance in patients with HFpEF.

Study rationale and objective

This study aims to assess the impact of accelerated pacing with tailored AV-regulation on the PCWP during exercise in patients with HFpEF, particularly at lower intensities of exertion, which are most relevant for daily activities. By determining whether accelerated pacing improves PCWP during exertion, we aim to provide insights into novel pacing-based interventions that could enhance exercise tolerance and ultimately improve quality of life in patients with HFpEF.

Risk assessment

When performed in experienced centers, right heart catheterization (RHC) is generally considered a safe procedure with low morbidity and mortality rates. A comprehensive analysis of 7,218 procedures, including both retrospective (5,727 cases) and prospective (1,491 cases) data, found an overall serious adverse event rate of 1.1% (95% CI: 0.8%–1.3%). The most frequent complications were related to venous access, such as hematoma and pneumothorax, followed by arrhythmias and hypotensive episodes due to vagal reactions or pulmonary vasoreactivity testing. The vast majority of these complications were mild to moderate in intensity and resolved spontaneously or with appropriate intervention (29). More severe complications are rare. Complete heart block or air embolism can lead to chest pain, shortness of breath, and hypotension. Pulmonary artery perforation, seen in approximately 0.03% of cases, may cause sudden respiratory distress and cardiogenic shock. In these cases, complications may require advanced medical intervention (30).

Radial artery canalization is generally well tolerated; complications are uncommon and include, but are not exclusive to, temporary or permanent vessel occlusion, pseudoaneurysm formation, infection, and, rarely, air embolism. Local pain at the site of cannulation, however, is common and may persist for days (31).

Potential complications of CPET include fatigue, leg pain and shortness of breath. Less common complications include cardiac arrhythmias, anginal chest pain, and bronchospasm. Additionally, CPET may lead to hypotension, occasional premature ventricular contractions (PVCs), and, in rare cases, significant ST-segment depression, which typically resolve after test termination. Overall, CPET remains a safe procedure with a low incidence of complications when conducted under medical supervision (32, 33).

2 Study objectives & design

2.1 Study objectives

In this study, we plan to address the impact of accelerated pacing with AV-regulation on the PCWP at rest and during exercise in patients with HFpEF. We hypothesize that accelerated pacing with AV-delay optimization reduces PCWP at rest and during exercise up to an optimal pacing rate beyond which no further benefit is observed.

In this study, we aim to:

- Evaluate the effect of accelerated pacing with AV-optimization on PCWP at rest.
- Investigate the impact of accelerated pacing with dynamic AV-delay programming, while avoiding truncation, on PCWP during exercise at low intensity (25 W) using a semi-supine cycle ergometer.
- Assess the change in CO, SV and peripheral oxygen extraction at the pacing frequency corresponding to the lowest PCWP compared to the baseline HR. This approach allows examination not only of the effects of pacing on backward cardiac function (as reflected by PCWP), but also on forward cardiac function. By doing so, it excludes the possibility that a reduction in PCWP is merely due to a decreased CO.
- Evaluate the effects of different pacing frequencies on SWV as a non-invasive marker of myocardial stiffness

Ultimately, we aim to offer insights into novel pacing-based interventions to enhance exercise tolerance and quality of life in patients with HFpEF.

2.2 Primary endpoints

The primary endpoints are:

- The change in PCWP at each pacing frequency (+20 bpm, +40 bpm, +60 bpm) with its corresponding optimal AV-delay at rest, compared to baseline PCWP.
- The change in PCWP at each pacing frequency (+10 bpm, +30 bpm, +50 bpm) using dynamic AV-delay programming, while avoiding truncation, compared to baseline PCWP during exercise (cycle ergometry at 25 W)

Baseline PCWP is calculated as the average of the measurements before and after each accelerated pacing-on step, using the intrinsic HR or baseline atrial pacing rate as the reference HR, as applicable.

2.3 Secondary endpoints

The secondary endpoints are:

- The change in CO at the pacing frequency corresponding to the greatest reduction in PCWP compared to the baseline HR
- The change in SV at the pacing frequency corresponding to the greatest reduction in PCWP compared to the baseline HR
- The change in peripheral oxygen extraction at the pacing frequency corresponding to the greatest reduction in PCWP compared to the baseline HR
- The change in SWV for each pacing frequency. The SWV at the intrinsic HR is defined as the average of the SWV at the baseline HR before and after each pacing step.

Cardiac output is measured via the direct Fick principle, dividing VO_2 by the peripheral oxygen extraction (SaO2-SvO2). SV is calculated as CO divided by HR. The baseline HR is defined as the intrinsic HR or baseline atrial pacing rate, as applicable.

2.4 Study design

This is a **prospective interventional** study, as it actively adjusts pacing rate and AV-delay in real time to assess their acute effects on PCWP at rest and during exercise.

The study is a **single arm** study. All patients undergo the pacing protocol (Figure 3 and 4) as an internal pairwise comparison is made between different pacing conditions and the baseline HR.

The study is **unblinded**. All measurements will be performed bedside, allowing patients to follow the different steps of the protocol. As a result, they will naturally become aware of pacing adjustments, making partial blinding unfeasible. While this may introduce some expectation bias in subjective measures such as palpitations or exertion intolerance, the primary (PCWP) and secondary endpoints (CO, SV, peripheral oxygen extraction, SWV) are objective physiological measures. Therefore, the absence of blinding does not compromise the validity of the results.

The study is **multicenter**, as both UZ Leuven and Jessa Hospital will recruit patients and implement the study protocol.

2.5 Expected duration of the study

Expected overall study duration for the research site

We expect the overall study duration to be **one year**. UZ Leuven currently follows 274, Imelda Hospital 150 patients and Jessa Hospital 400 patients with left bundle branch area (LBBA) pacing. As our goal is to include 20 patients, approximately 2% of all patients with LBBA pacing will need to consent to participate in the study. This percentage will be even higher after excluding patients who do not meet the eligibility criteria. Therefore, to achieve our target sample size of 20 patients, we anticipate needing to approach nearly all eligible patients who attend their regular (at least) **annual** pacemaker or heart failure consultation. After screening at their annual pacemaker consultation, we aim to plan the study visit within three weeks.

Expected overall study duration for a single participant

For individual participants, the study duration is expected to be three weeks. After providing informed consent, we aim to schedule the study visit within this timeframe. The total study visit is expected to take 2 hours. After the study visit, the study ends.

The End of Study (EoS) is defined as Last Participant Last Visit (LPLV). The Sponsor-Investigator shall notify the EC of the end of the study. The Sponsor-Investigator will submit a final report with the results of the study, including any publications/abstracts, to the Competent Authorities (CA)/Ethics Committee (EC) within 1 year of study termination or within 6 months for paediatric studies.

The Sponsor or EC can decide to halt or prematurely terminate the study when new information becomes available whereby the rights, safety and well-being of study participants can no longer be assured, when the integrity of the study has been compromised, or when the scientific value of the study becomes obsolete and/or unjustifiable.

Circumstances requiring premature treatment interruption or discontinuation of the study, include but are not limited to:

- Safety concerns or unacceptable intolerability

Should the study be temporarily suspended or, ended prematurely, the Sponsor-Investigator will notify the EC and include the reasons for suspension/premature termination within 15 days of the decision.

3 Study population / Eligibility criteria

3.1 Inclusion criteria

Participants eligible for inclusion in this study must meet **all** of the following criteria:

1. Voluntary written informed consent of the participant has been obtained prior to any screening procedures
2. At least 18 years of age at the time of signing the Informed Consent Form (ICF)
3. Heart failure with preserved ejection fraction, defined as one of the below criteria:

a. EF ≥ 45% and HFA-PEFF score ≥ 5 (Heart Failure Association–Pre-test assessment, Echocardiography and natriuretic peptide, Functional testing, Final etiology; heart failure association of the European Society of Cardiology and the Heart Failure Association (34))

b. EF ≥ 45% and H2FPEF score ≥ 6 (Heavy, Hypertensive, Atrial fibrillation, Pulmonary hypertension, Elder, Filling pressure; Mayo Clinic group (35)))

c. EF ≥ 45% and
   a. History of heart failure hospitalization after pacemaker implantation or
   b. On loop-diuretics at time of inclusion

4. Having a DDD-pacemaker with LBB area pacing, implanted at least 12 weeks before the invasive cardiopulmonary exercise test (iCPET)
5. ≥6 weeks on optimal HFpEF therapy (MRA and SGLT2i) at time of iCPET, unless contraindicated or not tolerated
6. Sinus rhythm or atrial paced rhythm at time of screening and iCPET

All participants that are considered for study participation, per the above criteria will be documented via applicable log forms in Investigator Site File (ISF) (including screen failures).

Figure 1: HFA-PEFF score

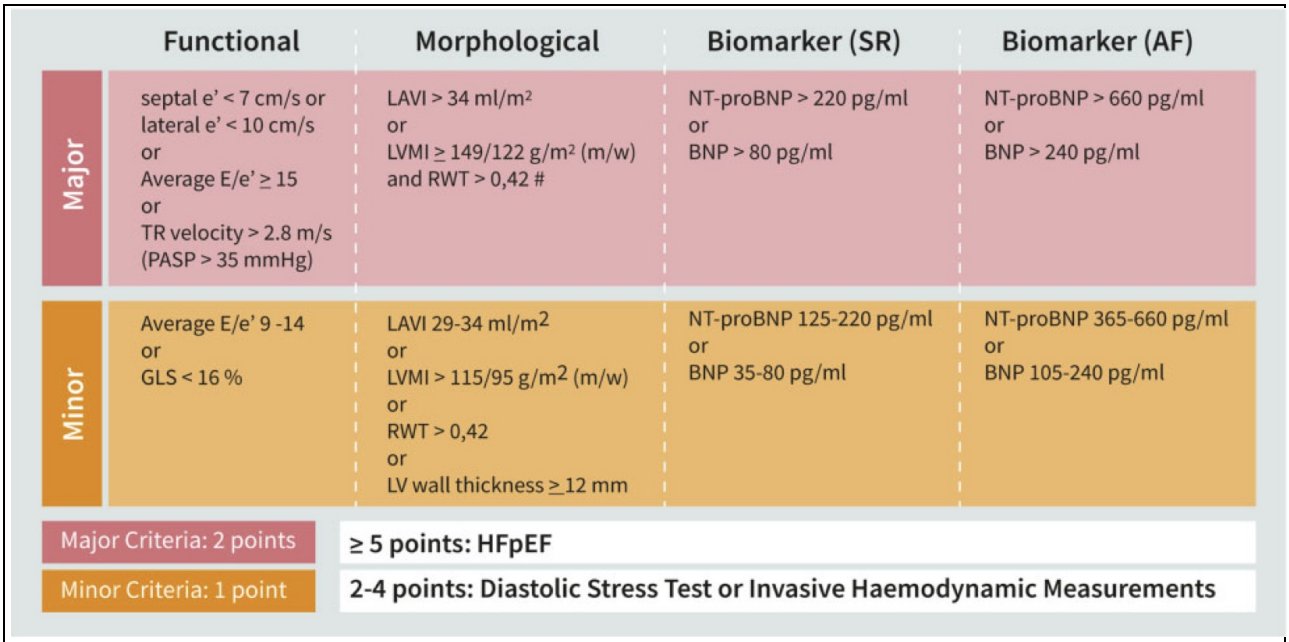

Figure 1: The HFA-PEFF score. Adapted from Pieske B, et al. European Heart Journal. 2019;40(40):3297-317. Abbreviations: AF, atrial fibrillation; BNP, B-type natriuretic peptide; GLS, global longitudinal strain; HFpEF, heart failure with preserved ejection fraction; LAVI, left atrial volume index; LV, left ventricular; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PASP, pulmonary artery systolic pressure; RWT, relative wall thickness; SR, sinus rhythm; TR, tricuspid regurgitation.

Figure 2: H2FPEF score

	Clinical Variable	Values	Points
H_2	Heavy	Body mass index > 30 kg/m^2	2
	Hypertensive	2 or more antihypertensive medicines	1
F	Atrial Fibrillation	Paroxysmal or Persistent	3
P	Pulmonary Hypertension	Doppler Echocardiographic estimated Pulmonary Artery Systolic Pressure > 35 mmHg	1
E	Elder	Age > 60 years	1
F	Filling Pressure	Doppler Echocardiographic E/e' > 9	1
H_2FPEF score			Sum (0-9)

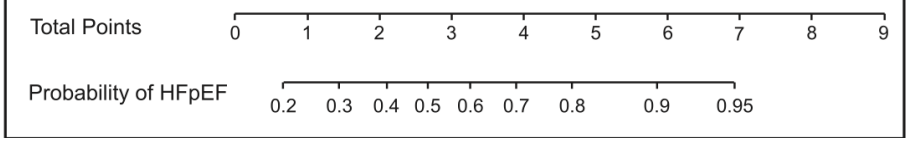

Figure 2: The H2FPEF score. Adapted from Reddy YNV, et al. Circulation. 2018;138(9):861-70.

3.2 Exclusion criteria

Participants eligible for this Study must **not** meet any of the following criteria:

1. Participant has a history of:
    a. Evidence of significant pulmonary comorbidity based on abnormal pulmonary function tests (FEV1 <60%) or aberrant lung parenchyma more than mild on radiological imaging
    b. Severe/symptomatic valvular diseases
    c. Severe pulmonary hypertension (PASP > 55mmHg estimated by Doppler Echo)
    d. Unstable arrhythmias (VT, VF)
    e. Recurrent syncopes after pacemaker implantation
    f. Permanent atrial fibrillation
    g. Amyloid cardiomyopathy
    h. History of reduced EF (EF < 45%)
2. Physical inability to perform exercise
3. More than 1 hospitalization for heart failure in the last year
4. Resting HR > 100bpm
5. At time of iCPET or inclusion: decompensated heart failure, unstable coronary syndrome
6. Contraindication to central venous access
    a. Severe coagulopathy (e.g., spontaneous INR > 2, thrombocytopenia < 50,000/µL)
    b. Local infection or skin infection at the insertion site
    c. Thrombosis or anatomical abnormalities of the right jugular vein
    d. Pneumothorax or contralateral lung pathology
    e. Inability to properly position the patient

7. Contraindication to arterial access
    a. Thrombosis or occlusion of the target artery
    b. Raynaud's phenomenon or other vasospastic disorders
    c. Active infection at the intended insertion site
    d. Severe coagulopathy (e.g., spontaneous INR > 2, thrombocytopenia < 50,000/µL)
    e. Insufficient collateral circulation (e.g., inadequate perfusion in an Allen test)
8. Contraindications to CPET
    a. ECG signifying myocardial injury
    b. ECG signifying current or potentially lethal arrhythmias
    c. Systemic hypotension (e.g. systolic blood pressure (BP) < 90 mmHg)
    d. Extreme hypertension (e.g. systolic BP > 220 mmHg)
    e. Syncope, presyncope, or lightheadedness
    f. SaO2 < 88%
    g. Severely elevated PCWP (> 40 mmHg) during exercise

Participants who meet one or more of the above exclusion criteria **must not proceed** to be enrolled/randomized in the study and will be documented as a screen failure via applicable log forms in Investigator Site File.

4 Study procedures

4.1 Selection of participants / Recruitment

Potential participants will be identified and recruited during their (at least) annual pacemaker or heart failure follow-up consultations at one of the participating sites and additionally at the Imelda Hospital, which will serve solely as a recruitment site. Informed consent and all subsequent study visits for patients recruited there will take place at UZ Leuven. Patients will be informed about the opportunity to take part in the study. If a patient expresses interest in participating, the informed consent documents will be provided. Participants will receive clear and comprehensive information, in understandable language, about the study's purpose, procedures, and any potential constraints. They will also be informed of their right to decline participation or withdraw from the study at any time without consequences. Any questions can be addressed immediately during the consultation, or later via email or phone (contact information will be provided). No additional recruitment material (such as for example flyers, social media advertising, audio/video recordings) will be used.

4.2 Study assessments & procedures

Study procedures and their timing are summarized in the Study flowchart.

4.2.1 Participant consent & withdrawal of consent

The Study will be conducted only on the basis of prior informed consent by the Study participants and/or their legally authorized representative(s). As such, no study-related procedures will be conducted prior to obtaining written informed consent from potential study participants.

The process for obtaining and documenting initial and continued informed consent from potential study participants will be conducted in accordance with Good Clinical Practice (GCP), applicable regulatory requirements and internal Standard Operating Procedures (SOPs).

All originally signed obtained Informed Consent Forms (ICFs) must be retained/archived in the Investigator Site File (ISF) at the participating site and must not be destroyed (even when a scanned

copy is available) before expiration of the legal archiving term as defined in the protocol section entitled "Archiving".

Participants may voluntarily withdraw consent to participate in the study for any reason at any time. The participant's request to withdraw from the study must always be respected without prejudice or consequence to further treatment. Consent withdrawal will be documented in the participant's medical record.

Study data and samples collected before withdrawal can be used in the study. No new study data or samples will be collected after withdrawal of the participant.

4.2.2 Other study assessments & procedures

4.2.2.1 Study specific and other procedures/investigations:

Additional procedures needed for screening

All patients known to receive left bundle branch area (LBBA) pacing at the time of ethics committee approval will be screened for eligibility by reviewing their medical records in KWS (n = 274 + n = 150 + n = 400). Eligible patients, based on the already available data, will be approached during their annual consultation.

If they consent to participate in the study, additional information will be collected if not already available:

- Recent transthoracic echocardiography (≤ 3 months), with the following parameters:
  - Ejection fraction
  - Septal e', lateral e', average E/e' or TR velocity
    - If septal e' ≥ 7cm/s or lateral e' ≥ 10cm/s or TR velocity ≤ 2.8 m/s: GLS
  - LAVI or LVMI and RWT
    - If LAVI ≤ 34 ml/m^2 or LVMI < 149/122 g/m^2 or RWT ≤ 0,42: LV wall thickness
  - Doppler echocardiographic estimated pulmonary artery systolic pressure
- Recent blood test with the following (≤ 3 months):
  - Thrombocyte count, INR
  - NT-proBNP
  - HbA1c
- ECG at time of consultation
- Weight and length at time of consultation
- BP at time of consultation
- NYHA classification at time of consultation
- Exercise stress echocardiography will be scheduled in patients with an HFA-PEFF score of 2-4, with measurement of the following:
  - Average E/e' at peak stress
  - Peak TR velocity

When meeting all eligibility criteria, patients will be contacted to schedule the iCPET (study visit).

The study protocol

Eligible patients will undergo one protocol at rest and one protocols in exercise as described below. The technical setup for iCPET with a cycle ergometer is already available at UZ Leuven and Jessa Hospital (S65913).

First, patients will be welcomed at the catheterization lab and receive a detailed explanation of the study protocol. A brief history taking and clinical examination will assure there are no signs of acute heart failure, nor unstable coronary syndrome. Then BP and peripheral oxygen saturation will be assessed non-invasively. Telemetry will confirm that the patient is in sinus rhythm or is being atrially paced. If all eligibility criteria are still met, the iCPET will proceed.

The interventional cardiologist will perform ultrasound-guided placement of a jugular venous catheter to allow insertion of a Swan-Ganz catheter and collection of venous blood gases for assessment of mixed venous oxygen saturation (SvO_2) and Haemoglobin. In addition, a radial artery catheter will be placed for arterial blood gas sampling to determine arterial oxygen saturation (SaO_2).

After catheter placement, the patient will be transferred to the cycle ergometer. Continuous monitoring of vital parameters and vascular access sites will be maintained throughout the transfer.

Upon arrival, the patient will be familiarized with the CPET mask, and cardiac electrodes will be attached. Continuous monitoring of vital parameters and vascular access sites will be maintained throughout each phase.

Pacing protocol in resting conditions

Subsequently, the study protocol in rest will begin (cfr. Figure 3). For a detailed description of the protocol we refer to appendix 3.

Figure 3: Pacing protocol in resting conditions

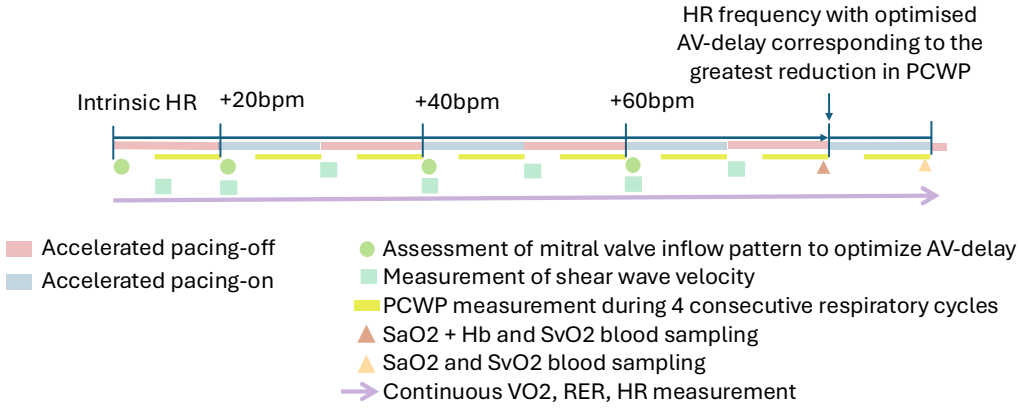

Figure 3: Pacing protocol in resting conditions. The average end-expiratory PCWP during 4 consecutive respiratory cycles will be included in our analyses. Stepwise increases in HR with optimized AV-delay will commence from the intrinsic HR rounded to the nearest decimal. If no reduction in PCWP is observed with accelerated pacing, the HR corresponding to the smallest increase in PCWP will be selected for the final pacing-on period instead. Abbreviations: AV, atrioventricular; Hb, hemoglobin; HR, heart rate; PCWP, pulmonary capillary wedge pressure; RER, respiratory exchange ratio; SaO2, arterial oxygen saturation; SvO2, mixed venous oxygen saturation; VO2, oxygen consumption.

Pacing protocol during exercise

Subsequently, the patient will be positioned on the semi-supine ergometer (45°), ensuring proper alignment and comfort. The patient will then start the cycling protocol at 25 W (cfr. Figure 4). For a detailed description of the protocol we refer to appendix 4.

Figure 4: Pacing protocol during exercise

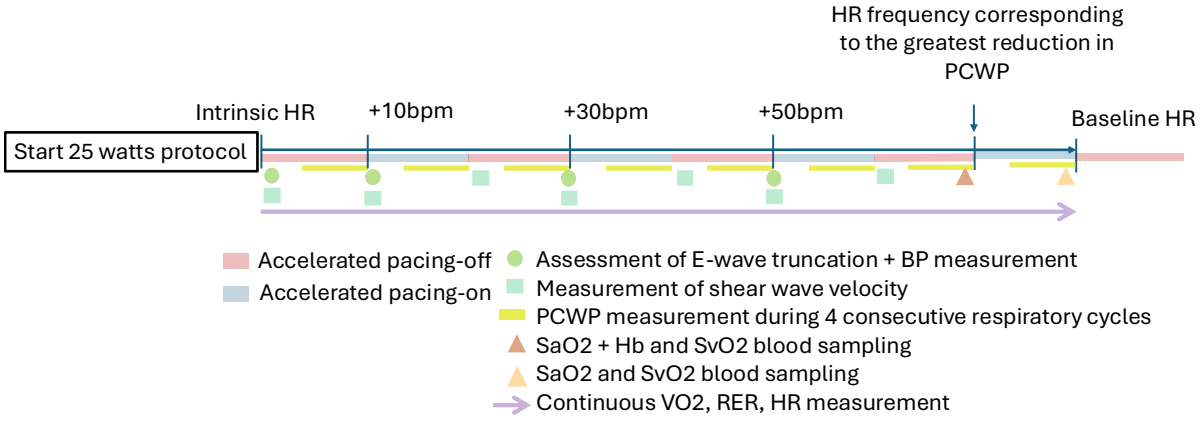

Figure 4: Pacing protocol during exercise. The exercise protocol will be performed at 25 W. The average end-expiratory PCWP during 4 consecutive respiratory cycles will be included in our analyses. Stepwise increases in HR with dynamic AV-delay settings will commence from the baseline HR rounded to the nearest decimal. If no reduction in PCWP is observed with accelerated pacing, the HR corresponding to the smallest increase in PCWP will be selected for the final pacing-on period instead. Abbreviations: AV, atrioventricular; Hb, hemoglobin; HR, heart rate; PCWP, pulmonary capillary wedge pressure; RER, respiratory exchange ratio; SaO2, arterial oxygen saturation; SvO2, mixed venous oxygen saturation; VO2, oxygen consumption.

End of study phase.

Upon completion of the exercise phase, the patient will cease cycling and remain in a semi supine position for a short recovery period. The right heart catheter and radial artery catheter will be carefully removed under sterile conditions. Hemostasis will be ensured by applying manual pressure or a hemostatic device as per standard protocol. The patient will then be assisted in sitting up and, if stable, allowed to leave the examination table. Vital signs will be monitored for a 15 minutes duration post-procedure, and any adverse events will be documented.

4.2.2.2 Laboratory tests and analysis:

Laboratory test	**Where and by whom is the sample collected?**	**Place of analysis** *(e.g. UZ Leuven LAG/CME/, Pathology, KU Leuven lab,, VIB, (other 3rd party lab….)*	**Other information** *(e.g. reference relevant SOP pertaining to this analysis)*
Blood test: including thrombocytes, INR, NT-proBNP, HbA1c	In and by UZ Leuven In and by Jessa Hospital	UZ Leuven LAG Jessa Hospital	
Arterial blood sampling: SaO2 and Hb Mixed venous blood sampling: SvO_2	In and by UZ Leuven In and by Jessa Hospital	UZ Leuven IRCC Jessa Hospital	

4.2.2.3 Location of the study:

The study will take place in UZ Leuven and in Jessa Hospital (Hasselt). Imelda Hospital (Bonheiden) will serve as a recruitment-only site.

4.3 Premature discontinuation of study

Participants may voluntarily discontinue from study treatment and/or prematurely end their participation in the study for any reason at any time. In such case the Investigator must make a

reasonable effort to contact the participant (e.g. via telephone and/or e-mail) in order to document the primary reason for this decision.

The Investigator may also decide at any time during the course of the study, to temporarily interrupt or permanently discontinue the study treatment if it is deemed that continuation would be detrimental to, or not in the best interest of the participant.

Circumstances requiring premature treatment interruption or discontinuation of the study, include but are not limited to:

- Study participation while in violation of the inclusion and/or exclusion criteria
- *If applicable*: Pregnancy
- *If applicable*: Intention of becoming pregnant

In any such case of early study termination and/or treatment interruption/discontinuation, the treating physician will continue to closely monitor the participant's condition and ensure adequate medical care and follow-up. It is recommended that follow-up information will be collected as follows:

No study visit will take place, however, standard clinical follow-up will proceed as previously scheduled.

For participants whose status is unclear because they fail to appear for study visits without stating an intention to discontinue or withdraw, the Investigator must make every effort to demonstrate "due diligence" by documenting in the source documents which steps have been taken to contact the participant to clarify their willingness and ability to continue their participation in the study (e.g. dates of telephone calls).

A participant should not be considered lost to follow-up until due diligence has been completed.

5 Safety reporting

5.1 Definitions

5.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or participant during an experiment, and which does not necessarily have a causal relationship with the treatment or intervention.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a product or intervention, whether or not considered related. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE.

Events that occur between the screening visit and the study visit will not be recorded as AEs or SAEs. Eligibility criteria will be reassessed at the study visit.

5.1.2 Adverse Reaction (AR)

An AR is any untoward and unintended response in a participant during an experiment in which a causal relationship between the study intervention and an adverse event is a reasonable possibility.

5.1.3 Serious Adverse Event (SAE)

An SAE is any unfavourable medical occurrence that results in any of the following:

- Inpatient hospitalisation or prolongation of existing hospitalization

- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- A life-threatening[a] experience
- Death
- Important medical events that may be considered an SAE when - based on appropriate medical judgement - they may jeopardise the participant and may require medical or surgical intervention to prevent one of the above outcomes

[a] *The term "life threatening" in the definition of SAE refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe.*

5.1.4 Serious Adverse Reaction (SAR)

A SAR is an adverse reaction that meets the criteria for a serious adverse event (see 5.1.3 Serious Adverse Event).

5.1.5 Adverse Events of Special Interest (AESI)

The following events should be reported within the same timelines as SAEs:

- Adverse events related during iCPET
  - ECG signifying myocardial injury
  - ECG signifying current or potentially lethal arrhythmias
  - Systemic hypotension (e.g. systolic BP < 90 mmHg)
  - Extreme hypertension (e.g. systolic BP > 220 mmHg)
  - Syncope, presyncope, or lightheadedness
  - SaO2 < 88%
  - Severely elevated PCWP (> 40 mmHg) during exercise
- Adverse events related to jugular catheter insertion and PCWP-measurement
  - Air embolism
  - Pulmonary artery perforation
  - Infection
  - Pneumothorax
  - Arrhythmias
  - Hypotension
- Adverse events related to arterial catheter insertion
  - Permanent vessel occlusion
  - Pseudoaneurysm formation
  - Infection
  - Air embolism

5.2 Adverse Events that do not require reporting

In general, the following should not be reported as AEs:

- Pre-existing conditions, including those found as a result of screening (these should be reported as medical history or concomitant illness). However, in case the pre-existing condition appears to be enhanced by the study intervention, this should be reported as an AE.

- Pre-planned procedures, unless the condition for which the procedure was planned has worsened from the first study-related activity after the participant has signed the informed consent.

The following events are commonly observed and are therefore not considered as adverse events for the purpose of the study:

- Delayed muscle onset pain
- Local pain at the site of cannulation
- Hematoma

Although these events should not be reported to the Sponsor, these should be recorded in the participant's medical notes according to routine practice.

The following events not to be considered as SAEs are:

- Pre-planned hospitalisations unless the condition for which the hospitalisation was planned has worsened from the first study-related activity after the participant has signed the informed consent.
- Hospitalisation as part of a standard procedure for protocol therapy administration. However, prolonged hospitalization or hospitalisation for a complication of therapy administration will be reported as an SAE.
- Hospitalisation or prolongation of hospitalisation for technical, practical, or social reasons, in absence of an AE.

5.3 Recording & reporting of Adverse Events

5.3.1 AE recording

Investigators will seek information on AEs during each participant contact. All events, whether reported by the participant or noted by study staff, will be recorded in the participant's medical record and in the eCRF within a reasonable time after becoming aware. The diagnosis rather than the individual signs or symptoms should be reported, if available. If no diagnosis is available, the Investigator should record each sign and symptom as individual AEs.

The following minimum information should be recorded for each (S)AR:

- AE description
- Start and stop date of the AE
- Severity
- Seriousness
- Causality assessment to the study interventions
- Outcome

5.3.2 Study safety risks & mitigation measures

The risks associated with the study specific interventions are:

- **Blood collection**: Venous blood samples are taken in a procedure that is not different from taking blood for other purposes in standard clinical care. Although taking blood from the vein is a safe procedure, it may cause slight discomfort and result in mild bruising, swelling or

redness. To minimize these risks, blood samples will be drawn by experienced health care provides using aseptic techniques and appropriate post-procedure care will be provided

- **Echocardiography:** Echocardiography is assessed in a procedure that is not different from doing an echocardiography for other purposes in standard clinical care. As a non-invasive imaging modality, echocardiography is considered a safe procedure with no known significant risks. Any potential discomfort due to prolonged probe contact will be minimized by ensuring appropriate patient positioning and communication during the procedure.
- **Stress test echocardiography:** stress echocardiography is performed using a semi-supine cycle and is routinely used for diagnostic purposes, considered safe when conducted under appropriate medical supervision. Potential risks are comparable to those associated with physical exertion and may include fatigue, shortness of breath, dizziness, or, rarely, cardiac arrhythmias or ischemic symptoms. To minimize these risks, patients will be carefully screened prior to testing, continuously monitored during the procedure (including electrocardiography and BP), and the test will be supervised by experienced healthcare professionals. The procedure will be terminated immediately if clinically significant symptoms or abnormalities occur.
- **Invasive CPET**: iCPET involves:
    - **Right heart catheterization and PCWP-measurements** in a procedure that is not different for other purposes in standard clinical care. Although RHC is regarded as a safe procedure when performed in experienced centers, it may cause venous access-related issues (hematoma, pneumothorax) as well as arrhythmias and hypotension due to vagal reactions. Severe complications (air embolism or pulmonary artery perforation) are rare. To mitigate the risk on complications, patients will undergo thorough medical evaluation excluding those with a history of unstable arrhythmias, severe tricuspid regurgitation or severe pulmonary hypertension, recurrent syncopes, or contra-indications for central venous access. Furthermore the procedure will be performed by an experienced interventional cardiologist, ultrasound guided and in sterile conditions, further limiting risks. A defibrillator will be available bedside in case of emergency.
    - **Radial artery cannulation** in a procedure that is not different for other purposes in standard clinical care. Placement of a radial artery cannula can cause slight discomfort and result in mild bruising, swelling or redness. Local anaesthesia will be used. While complications such as temporary or permanent vessel occlusion, pseudoaneurysm formation, infection, and, rarely, air embolism can occur, these events are uncommon. All risks will be limited due to the availability of an experienced interventional cardiologist, performing the cannulation in sterile conditions. An Allen test will be performed at study inclusion to make sure there is adequate collateral circulation through the ulnar artery, ensuring hand perfusion in case of radial artery occlusion. Patients will undergo thorough medical evaluation excluding those with contraindications for radial artery cannulation.

  Access sites will be monitored throughout the whole procedure. Both cannulas will be removed in sterile conditions. Hemostasis will be ensured by applying manual pressure or a hemostatic device as per standard protocol.
    - **CPET** on a semi supine cycle ergometer in a procedure that is not different for other purposes in standard clinical care. CPET is a widely used diagnostic tool that

is considered safe when conducted under medical supervision. However, potential complications can occur. Common symptoms include fatigue, leg pain, and shortness of breath. Less frequently, cardiac arrhythmias, anginal chest pain, and bronchospasm may develop. Rare complications include hypotension, occasional premature ventricular contractions (PVCs), and significant ST-segment depression, which typically resolve once the test is terminated. To mitigate the risk on complications, patients will undergo thorough medical evaluation excluding those with unstable coronary syndrome, decompensated heart failure, history of unstable arrhythmias, severe pulmonary hypertension and aortic stenosis. Throughout the procedure, a physician will monitor the participant to ensure safety and promptly manage any adverse events. An defibrillator will be available bedside in case of emergency.

- **Accelerated pacing on/off procedure:**

- During the study protocol, pacemaker settings will be actively adjusted to alternate between optimised baseline pacing settings and accelerated pacing at various frequencies. Previous studies involving stepwise increases in pacing rates in patients with HFpEF have all been conducted safely (26, 36-39). Nevertheless, accelerated pacing may pose certain physiological risks, including a reduction in diastolic filling time, potentially leading to diastolic underfilling and subsequent blunting of the Frank-Starling mechanism. These changes may contribute to hypotension and orthostatic symptoms (40). Pacing frequency will be limited to the age-predicted maximal HR (APMHR), preventing supraphysiological rates in patients with elevated resting HRs. Furthermore, abrupt transitions between accelerated pacing OFF and ON settings may cause palpitations, dyspnoea, or dizziness. To avoid excessive HR fluctuations, the lower rate during accelerated pacing–off episodes will be set ~20 bpm below the intrinsic HR at the corresponding exercise level. A minimum lower rate of 60 bpm (if exercise intrinsic HR <50 bpm) or 50 bpm otherwise will be maintained, instead of reverting to the resting lower rate. In patients with baseline atrial pacing, fixed atrial pacing rates of 80 bpm at 25 W will be applied. To mitigate potential risks, only patients in stable sinus or atrial paced rhythm will be included. All pacemaker adjustments will be performed under supervision of a cardiologist. Transitions between pacing modes will occur gradually, with continuous ECG and hemodynamic monitoring throughout. The study will take place in a fully equipped medical environment with a defibrillator and trained staff present at all times. In case of any signs of hemodynamic compromise, pacing will be immediately restored to a safe backup mode.

5.3.3 Assessment

All AEs must be evaluated by an Investigator as to:

- **Seriousness:** whether the AE is an SAE. See above for the seriousness criteria.
- **Severity:**

  Severity must be evaluated by an Investigator according to the following definitions:

  - *Mild* – no or transient symptoms, no interference with the participant's daily activities
  - *Moderate* – marked symptoms, moderate interference with the participant's daily activities
  - *Severe* – considerable interference with the participant's daily activities, unacceptable
- **Causality:**

- *None* – An AE which is not related to the study related interventions.
- *Unlikely* – An AE for which an alternative explanation is more likely (e.g. concomitant medication(s), concomitant disease(s)), and/or the relationship in time suggests that a causal relationship is unlikely.
- *Possible* – An AE which might be due to the study related interventions. An alternative explanation is inconclusive. The relationship in time is reasonable; therefore the causal relationship cannot be ruled out.
- *Probable* - An AE which might be due to study related interventions. The relationship in time is suggestive (e.g. confirmed by dechallenge). An alternative explanation is less likely.
- *Definitely* – An AE which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation. The relationship in time is very suggestive (e.g. it is confirmed by dechallenge and rechallenge).

5.3.4 Timelines for reporting

After informed consent has been obtained and after initiation of study related interventions:

All AEs and SAEs causally related to a study-related intervention and AESIs will be reported until 30 days after the last study-related intervention or until last follow-up visit (whichever occurs first).

All SARs and AESI as defined in the protocol must be reported to the Sponsor within 24 hours of the study staff becoming aware of the event. The immediate report shall be followed by detailed, written reports. The immediate and follow-up reports shall identify participants by study pseudonym.

SAR details will be reported by the Investigator to the Sponsor:

- By completing the SAE form in the eCRF
- By completing a paper SAE form and sending it to the following address in the event of a technical malfunction or if the eCRF does not have built-in notifications:

Mail: peter.sinnaeve@uzleuven.be

5.3.5 Follow-up

The Investigator must record follow-up information by updating the participant's medical records and the appropriate forms in the eCRF. A change in parameters (severity, seriousness, etc,) of the event will be recorded as a separate event .

SAE follow-up information should only include new (e.g. corrections or additional) information and must be reported within 24 hours of the Investigator's first knowledge of the information. This is also the case for previously non-serious AEs which subsequently become SAEs.

- All *SAEs* must be followed up until the outcome of the event is either 'recovered', 'recovered with sequelae', 'not recovered' (in case of death due to another cause) or 'death' (due to the SAE) and until all related queries have been resolved, or until end of study (whichever occurs first).
- *Non-serious AEs* must be followed up until the participant's last study visit, and until all related queries have been resolved.

SAEs after the end of the study: If the Investigator becomes aware of an SAE with suspected causal relationship to the study intervention or study participation after the participant has ended the study, the Investigator should report this SAE within the same timelines as for SAEs during the study.

5.3.6 Pregnancy

Female participants must be instructed to notify the Investigator immediately if they become pregnant during the Study. The Investigator must report any pregnancy to the Sponsor.

5.3.7 Death

Deaths of subjects who were participating in the study at the time of their death will be reported to the Sponsor without delay (irrespective of whether the death is related to disease progression, study procedure or is an unrelated event). The Sponsor will notify all deaths, as soon as possible after becoming aware, to the Central EC. Additionally, deaths occurring at a specific site will be reported to the EC of the concerned site, if requested by the respective EC.

5.4 Reporting requirements to Ethics Committee's (EC's)

The Investigator is responsible for ensuring that all safety events are recorded in the eCRF and reported to the Sponsor in accordance with instructions provided below.

The Sponsor will promptly evaluate all SARs and AESIs against medical experience to identify and expeditiously communicate possible new safety findings to the Investigators and to the EC(s) based on applicable legislation.

6.4.1 Annual reporting

The Sponsor has the obligation to, once a year throughout the study (or on request), submit a progress report to the ECs containing an overview of all SARs that occurred during the reporting period and taking into account all new available safety information received during the reporting period.

5.4.2 Overview reporting requirements

	WHAT	HOW	TO	TIMELINES
Investigator	AR	AE form in eCRF	Sponsor	In a timely manner
	SAR	SAE form in eCRF	Sponsor	Immediately (within 24 hours of becoming aware of the event) Exceptions: as defined in protocol
	death	SAE form in eCRF	Sponsor	asap
Sponsor	death	SAE form + narrative	ECs	asap
	Annual Progress Report	APR template	ECs	annually

5.4.3 Pharmacovigilance / Materiovigilance

Any incidents related to the use of a medical device should be reported to the Competent Authorities according to the materiovigilance guidelines.

6 Statistics and data analysis

A study-specific Statistical Analysis Plan (SAP) will be developed by the Sponsor, to supplement the information below with further details.

6.1 Sample size determination

The sample size calculation is based on the expected difference in PCWP between pacing conditions. A power analysis determined that 16 participants are required to achieve a power of 80% and a significance level of 5% (two-sided), assuming a mean difference of -2 mmHg and a standard deviation of 2.5 mmHg. To account for potential dropout, the study will enroll 20 participants.

6.2 Statistical analysis

This is a prospective, single-arm, multicentre, interventional study in which each patient serves as their own control:

- A two-sided significance level of 0.05 will be used for all statistical tests.
- Data will be analyzed using Python, SPSS and Stata statistical software.
- Descriptive statistics of baseline variables will be used to summarize baseline characteristics.
  - Categorical variables will be presented as number and percentage.
  - Continuous variables will be presented as mean ± standard deviation or median with interquartile range, depending on the Shapiro-Wilk test for normality.
- Comparison of hemodynamic parameters (PCWP, CO, SV, peripheral oxygen extraction) and SWV will be performed using paired student t-test or Wilcoxon-Mann Whitney test whichever will be appropriate.
- Pearson's or Spearman's correlation coefficients, whichever will be appropriate, will be computed to assess the relationship between changes in PCWP and changes in SWV

Endpoint	**Statistical analysis methods**
Primary	paired student t-test or Wilcoxon-Mann Whitney test
Secondary	paired student t-test or Wilcoxon-Mann Whitney test
Exploratory	descriptive statistics, Pearson's or Spearman's correlation coefficient

7 Data handling

A study-specific Data Management Plan (DMP) will be developed by the Sponsor providing a.o. further details on data flows, data handling, processing and discrepancy management.

7.1 Data collection tools and source document identification

7.1.1 Operational aspects

Data collection, handling, processing and transfer for the purpose of this study will be performed in compliance with applicable regulations, guidelines for clinical studies and internal procedures, as follows:

7.1.1.1 Data collection:

Source data will be collected and recorded in the study participant's files/medical records.

If applicable, worksheets may be used for capturing some specific data in order to facilitate completion of the eCRF. Any such worksheets will become part of the study participant's source

documentation and will be filed together with or as part of the medical records (during, and at the latest following completion of the study).

It remains the responsibility of the Investigator to check that all data relating to the study, as specified in the study protocol, are entered into the eCRF in accordance with the instructions provided and that the forms are filled out accurately, completely and in a timely manner.

eCRFs are provided by the Sponsor for each participant. The study data will be transcribed from the source records (i.e. participant's medical file or study-specific source data worksheets) into an eCRF by site study staff. Transcription to the eCRF will be done as soon as possible (preferably within 2 weeks of the participant's study visit, in order to allow for timely safety reviews by the Sponsor) and in a pseudonymized manner using a unique study-specific code assigned by the Sponsor.

Each participating research site shall maintain a log of the type of source documents and their location during and after completion of the study.

eCRF system:	REDCap Database
Questionnaire system:	/

The following data will be collected:	• Demographic variables
	• Clinical data (medical history, medication use, cardiovascular risk profile, weight, length, NYHA classification)
	• Echocardiographic variables:
	  ○ At time of inclusion: E, septal and lateral e', a', TR velocity, GLS, LAVI, LVMI, RWT, PASP
	  ○ During the study protocol: mitral valve inflow pattern (E, A wave), shear wave velocity
	• Stress echocardiography variables: peak TR velocity, peak average E/e'
	• Biomedical data: NT-proBNP, thrombocytes, INR, HbA1c
	• Raw time series CPET data, including VO_2, HR and RER
	• Data from pacemaker and ECG recording
	• Hemodynamic parameters: PCWP, non-invasive BP
	• Arterial and mixed venous samples: Hb, SaO_2 and SvO_2

7.1.1.2 Data validation:

All data relating to the study must be prepared and validated by the Investigator. Any eCRF entries, corrections and alterations must be made by the Investigator or other authorized study staff.

Proper audit trails must be available to demonstrate the validity of the study data collected. This includes historical records of original data entries, by whom and when the data was entered, as well as detailed records of any corrections or additions made to the original data entry (i.e. who made the correction/addition, when and why), without obliterating the original data entry information.

7.1.1.3 Data management:

The Study Data Manager will perform extensive logic and consistency checks on the received data. Queries will be issued in case of discrepancies in accordance with internal procedures.

7.1.1.4 Data transfer:

Any participant records or datasets that are transferred to the Sponsor or any partners of the Sponsor will contain the study-specific participant pseudonym only; participant names or any information which would make the participant identifiable will not be transferred. All pseudonymized data relating to the study must be transmitted in a secure manner to the Sponsor or any partners of the Sponsor (see 7.1.2. legal requirements).

7.1.2 Legal requirements

All source data will be kept at a secured location with restricted access at all times. These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data protection laws and regulations and more in particular the GDPR and relevant national laws implementing the GDPR. Appropriate technical and organizational measures to protect the data against unauthorized disclosure or access, accidental or unlawful destruction, or accidental loss or alteration must be established. Study staff whose responsibilities require access to personal data agree to keep the data confidential.

The Investigator and the participating site(s) (as applicable) shall treat all information and data relating to the study disclosed to them as confidential and shall not disclose such information to any third parties or use such information for any purpose other than the objectives of the study as described in this protocol. The collection, processing and disclosure of personal data, such as participant health and medical information is subject to compliance with applicable laws and regulations regarding personal data protection and the processing of personal data.

The Investigator will maintain all source documents and completed eCRFs that support the data collected from each study participant, and will maintain a Study Master File (TMF)/Investigator Site File (ISF) containing all study documents as specified in GCP (Appendix C) and as specified by applicable regulatory requirement(s).
The Investigator will take appropriate measures to prevent accidental or premature destruction of these documents.

Transfer of the pseudonymized data will be performed via a secured method of transfer taking into account all applicable security arrangements and regulations (such as the GDPR). The receiving party will be bound by contractual agreement to keep the transferred data confidential at all times and to only process the data for the purpose of the Study. To this end, additional appropriate Data Transfer Agreements (DTAs)may be established.

7.2 Audits and Inspections

The Investigator will permit direct access to study data and documents for the purpose of monitoring, audits and/or inspections by authorized entities such as but not limited to: the Sponsor or its designees and competent regulatory or health authorities. As such eCRFs, source records and other study related documentation (e.g. Investigator Site File, the Study Master File, etc.) must be kept current, complete and accurate at all times.

7.3 Monitoring

In accordance with GCP the Sponsor is responsible for monitoring the study to ensure compliance with GCP and current legislation, and to verify, among other requirements, that proper written informed consent has been obtained and documented, that the study procedures have been followed in accordance with the approved protocol, and that relevant study data have been collected and reported in a manner that assures data integrity.

According to UZ/KU Leuven policy, monitoring by the UZ/KU Leuven Clinical Trial Center (CTC) is not applicable for this study without IMP/IMD. Hence, based on this policy and as permitted by GCP, the Sponsor of the study accepts the minimal risks associated with this study and determines that monitoring activities (as defined by GCP) by a qualified individual, independent of the study team, is not necessary as it will provide little or no added value in protecting the safety of study participants and assuring the integrity of collected study data. Nonetheless, the Sponsor-Investigator study team will take all possible measures to assure the quality and integrity of study data and to safeguard the safety and wellbeing of study participants, in accordance with the requirements set out in GCP.

7.4 Archiving

As specified in GCP, the Sponsor and Investigator/participating site(s) will maintain a record of the location(s) of all respective essential study documents (including but not limited to source documents, completed and final eCRF and ISF/TMF). The Sponsor should ensure that the Investigator has control of and continuous access to the eCRF data reported to the sponsor during the study.

The Investigator/participating site should have control of all essential study documents and records generated by the Investigator/participating site before, during and following termination of the study.

Study-specific documentation (such as but not limited to the study protocol, any modifications thereto, the final Clinical Study Report (CSR) and the study database), as well assource data and site-specific study documents (such as but not limited to the original signed ICFs) will be archived by the participating site(s) according to local practice, and for at least 25 years following termination of the study. Archived data may be held on electronic record, provided that media back-up exists, hard copies can be obtained if required, and measures are taken to prevent accidental or premature loss or destruction of data. Destruction of essential study documents prior to, during or upon completion of the required archival period, will require written authorisation from the Sponsor.

8 Ethical & regulatory considerations

8.1 EC review & reports

Before the start of the study, this protocol and other related documents (e.g. ICF, advertisement and/or recruitment materials, Investigator Brochure (IB), etc.) will be submitted for review to the EC for approval. The study shall not commence until such approvals have been obtained and, until other relevant essential study documents, such as duly signed contract agreements, evidence of adequate study financing etc. are in place. In accordance with Sponsor policies, UZ/KU Leuven's Clinical Trial Center (CTC) will authorize study start-up (by means of "activating" the study) when all the above are available.

It is the responsibility of the Sponsor-Investigator to produce the Annual Progress Report (APR) and submit it to the EC within 30 days of the annual anniversary date on which favourable opinion to conduct the study was given, until the study is declared to have ended.

The Sponsor-Investigator shall notify the EC of the end of the study. Should the study be temporarily suspended or, ended prematurely, the Sponsor-Investigator will notify the EC and include the reason(s) for suspension/premature termination within 75 days of the decision. The Sponsor-Investigator will submit a final report with the results of the study, including any publications/abstracts, to the EC within 1 year of study termination.

8.2 Regulatory compliance

The study will be conducted in compliance with ICH-GCP E6(R3) guidelines, other GxP guidelines, the most recent version of the Declaration of Helsinki, the Belgian law of May 7th 2004 regarding experiments on the human person (as amended) and with the EU General Data Protection Regulation 2016/679 (GDPR), the relevant Belgian laws implementing the GDPR, the Belgian Law of August 22nd 2002 on patient rights and all other applicable legal and regulatory requirements.

8.3 Protocol / GCP compliance

The study will be performed in accordance with the protocol, current ICH and GCP guidelines, and applicable regulatory and country-specific requirements. ICH guidelines are an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of study participants are protected, consistent with the principles that originated in the most recent version of the Declaration of Helsinki, and that the study data are credible, reliable and reproducible.

The Investigator and study team acknowledge and agree that prospective, planned deviations or waivers to the protocol are not permitted under applicable regulations on clinical studies. However, should there be an accidental protocol deviation, such deviation shall be adequately documented in the source documents and on the relevant forms and promptly reported to the Sponsor/Sponsor-Investigator. Deviations should also be reported to the EC as part of the EC's continued review of the study (e.g. through the annual safety report (ASR), annual progress report (APR), etc.). Protocol deviations which are found to frequently recur, will require (immediate) action. The Investigator acknowledges that such recurring protocol deviations could potentially be classified as a major deviation of ICH and/or the protocol.

It is understood that "a major deviation" is likely to affect to a significant degree:

- the safety or physical or mental integrity of the study participant(s); or
- the scientific validity of the study

The Investigator is expected to take any immediate action required to protect the safety of any participant included in the study, even if this action represents a deviation from the protocol. In such cases, the Sponsor should be notified of this action and the EC at the study site should be informed according to local procedures and regulations.

8.4 Data protection and participant confidentiality

The study will be conducted in compliance with the requirements of the GDPR, the relevant Belgian laws implementing the GDPR including the Belgian Law of July 30th 2018 on the protection of natural persons with regard to the processing of personal data. Any collection, processing and disclosure of personal data, such as participant health and medical information is participant to compliance with the aforementioned personal data protection laws (cfr. Data Processing Annex (DPA) in Appendix). In case personal data is transferred outside the European Economic Area, safeguards will be taken to ensure that appropriate protection travels with the data in accordance with the GDPR.

Any personal data shall be treated as confidential at all times including during collection, handling and use or processing, and the personal data (including in any electronic format) shall be stored securely at all times and with all technical and organizational security measures that would be necessary for compliance with EU and national data protection legislation (whichever is more

stringent). The Sponsor shall take appropriate measures to ensure the security of all personal data and guard against unauthorized access thereto or disclosure thereof or loss or destruction while in its custody.

8.5 Insurance

The participating site, the Investigator and Sponsor shall have and maintain in full force and effect during the term of this study, and for a reasonable period following termination of the study, adequate insurance coverage for: (a) medical professional and/or medical malpractice liability, and (b) general liability.

(i) ***For Belgian participating sites***

Art 29 of the Belgian Law relating to experiments on human persons dated May 7th, 2004 applies.

Prior to the start of the study, the Sponsor shall enter into an insurance contract in order to adequately cover study participants from Belgian sites in accordance with art. 29 of the said law.

(ii) ***For non-Belgian participating sites (if applicable)***

The participating site shall have and maintain in full force and effect during the term of this study (and for a reasonable period following termination of the study, adequate insurance coverage for other possible damages resulting from the study at the participating site, as required by local law. Each such insurance coverage shall be in amounts appropriate to the conduct of the services of the participating site under this study.

The participating site and Sponsor shall be solely responsible for any deductible or self-insured retention under any such policies.

8.6 Modifications

During the study the Sponsor may modify the study. It is the Sponsor's responsibility to assess whether a modification is substantial or non-substantial.

A "substantial modification" is defined as any change of the study which is made after a decision is issued on a previously submitted application and that is likely to 'substantially impact the participants' safety or rights or the reliability and robustness of the data generated in the study. A substantial modification may only be implemented if it has been approved by the EC with no prejudice to the right of Sponsor and the Principal Investigator to take urgent safety measures without awaiting prior authorisation. In that case, the event and the measures taken should be reported to the EC as soon as possible. The EC will provide a response in accordance with timelines defined by applicable regulations.

A "non-substantial modification" is any change outside the scope of a substantial modification and irrelevant to the supervision of the study. A non-substantial modification should not be notified as such. These changes should be implemented during the next substantial modification. Non-substantial modifications need to be listed and identified as such in the cover letter of the substantial modification application to EC.

9 Research registration, dissemination of results & publication

The Declaration of Helsinki (latest version) and European and Belgian regulations require that every study involving human participants be registered in a publicly accessible database before

recruitment of the first participant. The Sponsor-Investigator is responsible for registering the study.

In addition, the Sponsor-Investigator will fulfil its ethical obligation to disseminate and make the research results publicly available. As such the Sponsor-Investigator is accountable for the timeliness, completeness and accuracy of the reports. Researchers, authors, sponsors, editors and publishers must adhere to accepted guidelines for ethical reporting. Negative and inconclusive, as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in publication.

Publications will be coordinated by the Sponsor-Investigator. Authorship to publications will be determined in accordance with the requirements published by and in accordance with the requirements of the International Committee of Medical Journal Editors.

For multicentre studies, it is anticipated that the primary results of the overall study shall be published in a multicentre publication.

Participating sites are not allowed to publish any subset data or results from the study prior to such multicentre publication.

Any publication by a participating site must be submitted to the Sponsor for review at least thirty (30) calendar days prior to submission or disclosure. Sponsor shall have the right to delay the projected publication for a period of up to three (3) months from the date of first submission to the Sponsor in order to enable the Sponsor to take steps to protect its intellectual property rights and know-how.

10 Intellectual property

Any know how, inventions, methods, developments, innovations, discoveries and therapies, whether patentable or not, arising from the study or made in the performance of the study protocol ("Inventions") shall vest in the Sponsor. The participating site, its employees and Investigator(s) shall promptly disclose to the Sponsor any such inventions. Parties have expressly agreed that any and all study data as collected and prepared in the performance of the study protocol shall be the sole property of Sponsor unless otherwise agreed in a clinical study agreement.

11 Quality

In order to ensure the same quality and safety standards in participant care for clinical research as commonly applied by the Sponsor in its regular activities, the Sponsor shall comply with the following obligations: (a) the Sponsor will use trained and qualified employees or contractors to manage and coordinate the study; (b) the Sponsor will ensure that (multi-center) study reporting is reliable and valid, statistically accurate, ethical, and unbiased. (c) the Sponsor will not grant incentives, other than standard compensations and reimbursement of costs, to study participants or to participating site's staff that would compromise the integrity of the research; (d) the Sponsor is responsible for monitoring and evaluating the quality, safety, and ethics of the study and will respect the participating site's policies and processes when performing such monitoring and evaluation activities; (e) the Sponsor will protect the privacy and confidentiality of the study participants in accordance with all applicable laws.

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APPENDICES

APPENDIX 1: Research protocol history

APPENDIX 2: Data processing annex

APPENDIX 3: Detailed description of the study protocol in resting conditions

APPENDIX 4: Detailed description of the study protocol in during exercise

13 APPENDIX 1: Research protocol history

Original protocol version:	3.0 dated 26/08/2025

Amendment #1:	4.0 dated 24/03/2026
Modifications made / Reason for amendment:	
Study Synopsis	• The study number in the public database has been completed. • The Endpoint section has been revised: comparisons are now made against baseline HR instead of intrinsic HR, allowing inclusion of patients with an atrially paced rhythm.
Study Flowchart	• For UZ Leuven, a potential second visit was added, allowing a stress echocardiography to be scheduled if needed. For Jessa, this was added as a potential third screening visit. • Visit windows were adjusted to allow greater flexibility due to challenging scheduling. • At Jessa, the second screening visit has been changed to consistently present. During this visit, the investigator invites patients who indicated interest in participating and are potentially eligible based on screening Visit 1 to receive study information, complete the informed consent form, and have demographics, medical history, physical exam, BP, and weight/height and echocardiography re-assessed due to potential delays from the initial screening visit. These adjustments were made to provide more flexibility in scheduling for these patients.
Study Objectives & design	• Comparisons are now made against baseline HR instead of intrinsic HR, allowing inclusion of patients with an atrially paced rhythm.
Study Population	• The inclusion criterion for ejection fraction (EF) has been revised to ≥45%. • Patients with an atrially paced rhythm are now specially mentioned as eligible for inclusion. • An episode of atrial fibrillation in the last 3 months is no longer an exclusion criterion. Permanent atrial fibrillation is now an exclusion criterion.
Study Procedures	• Exercise stress echocardiography has been added to the screening procedures
Safety Reporting	• Study safety risks associated with stress echocardiography have been added to the protocol. • Study safety risks associated with the accelerated pacing procedure have been updated to include patients with an atrially paced rhythm. References to (ventricular) back-up pacing have been removed; instead, the lower rate is now specified, as all patients have a DDD pacemaker.
Data Handling	• The data collection on stress echocardiography variables are added to the protocol

Appendix 3 & 4: Detailed description of the study protocol	• A detailed plan on the accelerated pacing protocol have been updated to include patients with an atrially paced rhythm. • References to (ventricular) back-up pacing have been removed; instead, the lower rate is now specified, as all patients have a DDD pacemaker. • Reference is made to accelerated pacing being turned on and off, rather than pacing itself being turned on and off, to make clear the pacemaker itself is never turned off.

Amendment #2:	5.0 dated 14-04-2026
Modifications made / Reason for amendment:	
Study Synopsis	The 10 W exercise stage is removed from the study protocol; the endpoint description has been updated accordingly.
Study objectives & Design	• The 10W exercise stage is removed from the study protocol description as two exercise stages required patients to sustain physical exertion for an excessively prolonged duration. (study objectives, primary and secondary endpoints) • The primary endpoint description has been rephrased for improved clarity and readability. No change to scientific content. • Imelda Hospital has been added to the study as a recruitment-only site. Informed consent and all study visits for patients recruited there will be conducted at UZ Leuven (expected duration of the study)
Study Population/Eligibility Criteria	• It has been clarified that patients must have been on optimal HFpEF therapy for ≥ 6 weeks at the time of the iCPET study visit as the original wording was ambiguous regarding the reference timepoint for the 6-week criterion (inclusion criteria) • It has been clarified that patients with recovered HFrEF are also excluded to avoid inclusion of patients whose cardiac function has recovered from a previously reduced ejection fraction, which would confound the HFpEF study population (exclusion criteria)
Study Procedures	• Imelda Hospital has been added to the study as a recruitment-only site. Informed consent and all study visits for patients recruited there will be conducted at UZ Leuven (selections of participants/recruitment) • Telemetry is sufficient to confirm sinus rhythm or atrially paced rhythm at the start of the study protocol (other study assessments & procedures) • The 10W exercise stage is removed from the study protocol description as two exercise stages required patients to sustain physical exertion for an excessively prolonged duration.
APPENDIX 3: Detailed description of the study protocol in resting conditions	• Section rewritten for clarity. Clarification of the timing of BP measurements

APPENDIX 4: Detailed description of the study protocol during exercise	• Section rewritten for clarity. Clarification of the timing of BP measurements • The 10W exercise stage is removed from the study protocol description as two exercise stages required patients to sustain physical exertion for an excessively prolonged duration.

14 APPENDIX 2: Data Processing Annex (DPA) between Sponsor and participating research site(s)

Definitions:

- "Protocol" means the document entitled Protocol for a prospective experiment: the impact of accelerated pacing and AV-delay regulation on the pulmonary capillary wedge pressure during exercise in patients with HFpEF containing the details of the academic study as developed by the Sponsor and approved by the relevant EC.
- "Sponsor" means UZ/KU Leuven (University Hospital Leuven/ KU Leuven).
- Participating site acts as a data processor as defined under article 4, 8) of the regulation (EU) 2016/679 ("Data Processor") for the Sponsor who acts as data controller as defined under article 4, 7) of the regulation (EU) 2016/679 ("Data Controller").
- "Applicable Law" means any applicable data protection or privacy laws, including:
  a) the regulation (EU) 2016/679 also referred as the General Data Protection Regulation ("GDPR");
  b) other applicable laws that are similar or equivalent to or that are intended to or implement the laws that are identified in (a) of this definition;
- "Personal Data" means any information relating to an identified or identifiable natural person ("Data Subject"), including without limitation pseudonymized information, as defined in applicable law and described in the protocol.

Rights and obligations:

1. The Data Processor is instructed to process the personal data for the term of the study and only for the purposes of providing the data processing tasks set out in the protocol. The Data Processor may not process or use personal data for any purpose other than the study, or other than provided in the instructions of the study protocol, including with regard to transfers of personal data to a third country or an international organization, unless the Data Processor is required to do so according to European Union or Member State law.
2. Data Processor shall at all times maintain a record of processing of personal data in accordance with applicable law and if the Data Processor considers an instruction from the Data Controller to be in violation of the applicable law, the Data Processor shall promptly inform the Data Controller in writing about this.
3. The Data Processor must ensure that persons authorized to process the personal data have committed themselves to confidentiality or are under an appropriate statutory obligation of confidentiality.
4. The Data Processor shall implement appropriate technical and organizational measures to prevent that the personal data processed is:
   (i) accidentally or unlawfully destroyed, lost or altered,
   (ii) disclosed or made available without authorization, or
   (iii) otherwise processed in violation of applicable law.
5. The appropriate technical and organizational security measures must be determined with due regard for:

(i) the current state of the art,

(ii) the cost of their implementation, and

(iii) the nature, scope, context and purposes of processing as well as the risk of varying likelihood and severity for the rights and freedoms of natural persons.

6. Taking into account the nature of the processing, the Data Processor shall assist the Data Controller, by means of appropriate technical and organizational measures, insofar as this is possible, in fulfilling its obligation to respond to requests from data subjects pursuant to laws and regulations in the area of privacy and data protection (such as, the right of access, the right to rectification, the right to erasure, the right to restrict the processing, the right to data portability and the right to object)

7. The Data Processor shall upon request provide the Data Controller with sufficient information to enable the Data Controller to ensure that the Data Processor's obligations under this DPA are complied with, including ensuring that the appropriate technical and organizational security measures have been implemented.

8. The Data Controller is entitled to appoint at its own cost an independent expert, reasonably acceptable to the Data Processor, who shall have access to the Data Processor's data processing facilities and receive the necessary information for the sole purpose of auditing whether the Data Processor has implemented and maintained said technical and organizational security measures. The expert shall upon the Data Processor's request sign a non-disclosure agreement provided by the Data Processor, and treat all information obtained or received from the Data Processor confidentially, and may only pass on, after conferral with the Data Processor, the findings as described under 10) (ii) below to the Data Controller.

9. The Data Processor must give authorities who by European Union or Member State law have a right to enter the Data Controller's or the Data Controller's processors' facilities, or representatives of the authorities, access to the Data Processor's physical facilities against proper proof of identity and mandate, during normal business hours and upon reasonable prior written notice.

10. The Data Processor must without undue delay in writing notify the Data Controller about:

(i) any request for disclosure of personal data processed under the protocol by authorities, unless expressly prohibited under European Union or Member State law,

(ii) any finding of (a) breach of security that results in accidental or unlawful destruction, loss, alteration, unauthorized disclosure of, or access to, personal data transmitted, stored or otherwise processed by the Data Processor under the protocol, or (b) other failure to comply with the Data Processor's obligations, or

(iii) any request for access to the personal data (with the exception of medical records for which the Data Processor is considered data controller) received directly from the data subjects or from third parties.

11. Such a notification from the Data Processor to the Data Controller with regard to a breach of security as meant in 10) (ii)(a) above will contain at least the following information:

(i) the nature of the personal data breach, stating the categories and (by approximation) the number of data subjects concerned, and stating the categories and (by approximation) the number of the personal data registers affected (datasets);

(ii) the likely consequences of the personal data breach;

(iii) a proposal for measures to be taken to address the personal data breach, including (where appropriate) measures to mitigate any possible adverse effects of such breach.

12. The Data Processor shall document (and shall keep such documentation available for the Data Controller) any personal data breaches, including the facts related to the personal data breach, its effects and the corrective measures taken. After consulting with the Data Controller, the Data Processor shall take any measures needed to limit the (possible) adverse effects of personal data breaches (unless such consultation cannot be awaited due to the nature of the personal data breach).

13. The Data Processor must promptly and reasonably assist the Data Controller (with the handling of (a) responses to any breach of security as described in 10) (ii) above and (b) any requests from data subjects under Chapter III of the GDPR, including requests for access, rectification, blocking or deletion. The Data Processor must also reasonably assist the Data Controller by implementing appropriate technical and organizational measures for the fulfilment of the Data Controller's obligation to respond to such requests.

14. The Data Processor must reasonably assist the Data Controller with meeting the other obligations that may be incumbent on the Data Controller according to European Union or Member State law where the assistance of the Data Processor is implied, and where the assistance of the Data Processor is necessary for the Data Controller to comply with its obligations. This includes, but is not limited to, at the request to provide the Data Controller with all necessary information about an incident under 10) (ii), and all necessary information for an impact assessment in accordance with Article 35 and Article 36 of the GDPR.

Subprocessor:

15. The Data Processor may only engage a subprocessor, with prior specific or general written consent from the Data Controller. The Data Processor undertakes to inform the Data Controller of any intended changes concerning the addition or replacement of a subprocessor by providing a reasonable prior written notice to the Data Controller. The Data Controller may reasonably and in a duly substantiated manner object to the use of a subprocessor. The Data Processor must inform the Data Controller in writing of the discontinued use of a subprocessor.

16. Prior to the engagement of a subprocessor, the Data Processor shall conclude a written agreement with the subprocessor, in which at least the same data protection obligations as set out in this DPA shall be imposed on the subprocessor, including obligations to implement appropriate technical and organizational measures and to ensure that the transfer of personal data is done in such a manner that the processing will meet the requirements of the applicable law.

17. The Data Controller has the right to receive a copy of the relevant provisions of Data Processor's agreement with the subprocessor related to data protection obligations. The Data Processor shall remain fully liable to the Data Controller for the performance of the subprocessor obligations under this DPA. The fact that the Data Controller has given consent to the Data Processor's use of a subprocessor is without prejudice for the Data Processor's duty to comply with this DPA.

15 APPENDIX 3: Detailed description of the study protocol in resting conditions

The following section provides a stepwise description of the pacing protocol performed at rest (Figure 3):

- Accelerated pacing-off:
  - Baseline settings: set the lower rate to 60 bpm if intrinsic HR < 50 bpm, otherwise to 50 bpm. Disable rate responsive pacing. Define baseline HR as the intrinsic HR (without atrial pacing) or 60 bpm (with atrial pacing), as applicable.
  - Optimize AV-delay
  - Measure non-invasive BP
  - 10 seconds after AV-delay optimization: measure SWV & PCWP
- Accelerated pacing-on:
  - Set the lower rate to **20** bpm above baseline HR, rounded to the nearest decimal
  - Optimize AV-delay
  - Measure non-invasive BP
  - 10 seconds after AV-delay optimization: measure SWV & PCWP
- Accelerated pacing-off:
  - Reprogram the pacemaker to optimized baseline settings
  - 10 seconds after reprogramming: measure SWV & PCWP
- Accelerated pacing-on:
  - Set the lower rate to **40** bpm above baseline HR, rounded to the nearest decimal
  - Optimize AV-delay
  - Measure non-invasive BP
  - 10 seconds after AV-delay optimization: measure SWV & PCWP
- Accelerated pacing-off:
  - Reprogram the pacemaker to optimized baseline settings
  - 10 seconds after reprogramming: measure SWV & PCWP
- Accelerated pacing-on:
  - Set the lower rate to **60** bpm above baseline HR, rounded to the nearest decimal
  - Optimize AV-delay
  - Measure non-invasive BP
  - 10 seconds after AV-delay optimization: measure SWV & PCWP
- Accelerated pacing-off:
  - Reprogram the pacemaker to optimized baseline settings
  - 10 seconds after reprogramming: collection of arterial and mixed venous blood samples
  - Measure SWV & PCWP
- Accelerated pacing-on
  - Set the lower rate to the HR corresponding to the greatest reduction in PCWP
  - 10 seconds after reprogramming: measure PCWP
  - After 4 respiratory cycles: collect arterial and mixed venous blood samples
- Accelerated pacing-off: reprogram to optimized baseline settings

HR, VO_2, RER and will continuously be measured.

The optimal AV-delay will be determined via iterative evaluation of the mitral valve inflow by transthoracic echocardiography. This will be followed by SWV measurements, performed over a 12-second period. PCWP will be measured as the average end-expiratory value over four consecutive respiratory cycles, following a 10-second period to allow for hemodynamic stabilization. By comparing each PCWP value to the average of the PCWP measured during the accelerated pacing-off periods immediately before and after the accelerated pacing-on phase, we aim to minimize the impact of spontaneous hemodynamic variability and carry-over effects (41). The HR associated with the greatest reduction in PCWP will be repeated as the final accelerated pacing-on step, during which CO will be measured by collecting arterial and mixed venous blood samples (direct Fick principle). If two pacing rates result in an equal reduction in PCWP, the lower pacing rate will be selected. If no reduction in PCWP is observed with accelerated pacing, the HR corresponding to the smallest increase in PCWP will be selected for the final accelerated pacing-on period instead. In this final step, PCWP will be measured again to verify that it remains consistent with the initial PCWP at the same pacing rate. Only the initial PCWP measurement at the optimal pacing rate will be used in our analyses assessing changes in PCWP compared to the baseline HR.

The pacing rate will never exceed the APMHR, calculated based on Tanaka's equation (42). If this threshold would be reached, the protocol will be stopped at the pacing step prior to exceeding the APMHR, only followed by the final pacing step in which the pacing rate associated with the greatest reduction in PCWP is repeated.

16 APPENDIX 4: Detailed description of the study protocol during exercise

The following section provides a stepwise description of the pacing protocol performed during exercise at 25 W (Figure 4):

- Accelerated pacing-off:
  - Baseline settings: set the lower rate to 80 bpm if the intrinsic HR is < 50 bpm or > 5 bpm below the baseline HR at rest; otherwise, set the lower rate to 50 bpm. If intrinsic HR > 70bpm: set lower rate to 20bpm below intrinsic HR.
  - Disable rate responsive pacing. Define baseline HR as the intrinsic HR (without atrial pacing) or 80 bpm (with atrial pacing), as applicable.
  - Evaluate the mitral inflow pattern on echocardiography for E-wave truncation; if observed, adapt AV-delay accordingly
  - Measure non-invasive BP
  - 10 seconds after reprogramming: measure SWV & PCWP
- Accelerated pacing-on:
  - Set the lower rate to **10** bpm above baseline HR, rounded to the closest decimal and with dynamic AV-delay settings
  - Evaluate mitral inflow pattern on echocardiography for evidence of E-wave truncation; if truncation is observed, adapt AV-delay accordingly.
  - Measure non-invasive BP
  - 10 seconds after reprogramming: measure SWV & PCWP
- Accelerated pacing-off:
  - Reprogram the pacemaker to the optimized 25W baseline settings

    - 10 seconds after reprogramming: measure SWV & PCWP
- Accelerated pacing-on:
    - Set the lower rate to **30** bpm above baseline HR, rounded to closest decimal and with dynamic AV-delay settings
    - Evaluate mitral inflow pattern on echocardiography for evidence of E-wave truncation; if truncation is observed, adapt AV-delay accordingly.
    - Measure non-invasive BP
    - 10 seconds after reprogramming: measure SWV & PCWP
- Accelerated pacing-off:
    - Reprogram the pacemaker to the optimized 25W baseline settings
    - 10 seconds after reprogramming: measure SWV & PCWP
- Accelerated pacing-on:
    - Set the lower rate to **50** bpm above baseline HR, rounded to the closest decimal and with dynamic AV-delay settings
    - Evaluate mitral inflow pattern on echocardiography for evidence of E-wave truncation; if truncation is observed, adapt AV-delay accordingly.
    - Measure non-invasive BP
    - 10 seconds after reprogramming: measure SWV & PCWP
- Accelerated pacing-off:
    - Reprogram the pacemaker to the optimized 25W baseline settings
    - 10 seconds after reprogramming: collect arterial and mixed venous blood samples
    - SWV & PCWP measurement
- Accelerated pacing-on:
    - Set the lower rate to the HR corresponding to the greatest reduction in PCWP
    - 10 seconds after reprogramming: measure SWV & PCWP
    - After 4 respiratory cycles: collect arterial and mixed venous blood samples
    - Stop cycling
- Accelerated pacing-off:
    - Reprogram the pacemaker to its original settings

The lower rate during accelerated pacing–off episodes will be set ~20 bpm below the intrinsic HR at the corresponding exercise level. A minimum lower rate of 60 bpm (if exercise intrinsic HR <50 bpm) or 50 bpm otherwise will be maintained, instead of reverting to the resting lower rate. In patients with baseline atrial pacing, fixed atrial pacing rates of 80 bpm at 25 W will be applied. During exercise, no optimal AV-delay will be determined; instead, dynamic AV-delay regulation will be applied and subsequently verified to ensure no truncation occurs. If truncation is detected, the AV-delay will be adjusted accordingly. Additionally, BP will be measured noninvasively. All other procedures and considerations are identical to those described for the protocol at rest.