

Renal Denervation in Heart Failure with Preserved Ejection Fraction (RDT-PEF): a randomised controlled trial

Supplementary Material Online

Eligibility Criteria

All patients recruited in this trial will have to HFpEF as detailed in the 2012 ESC guideline for heart failure diagnosis. It requires the following four conditions to be satisfied:

1. Symptoms typical of heart failure
2. Signs typical of heart failure (though this may not be present in diuresed patients)
3. Normal or only mildly reduced left ventricular (LV) ejection fraction (EF) and LV is not dilated
4. Relevant structural heart disease (LV hypertrophy/left atrial (LA) enlargement) and/or diastolic dysfunction

Further to the above the following criteria will be used to determine eligibility for the study:

Inclusion Criteria

- NYHA class II-III heart failure symptoms
- Non-dilated LV(LVEDD <60mm or LVESD <45mm)
- EF \geq 50% (revised to >40% on 31 October 2015)

- Either brain natriuretic peptide (BNP) >35ng/L or E/E' (lateral)>10 or E/E' (septal)>15
- Either dilated LA (diameter >38mm (women)/>40mm (men) or area > 20cm²) or LV hypertrophy (wall thickness ≥12mm) on echocardiography
- Heart failure hospitalisation in previous 24 months (removed on 31 October 2015)
- Age: 18-85 years
- Able to give informed consent

Exclusion Criteria

- Any form of cardiomyopathy (e.g. hypertrophic, restrictive, dilated) diagnosed
- Previously documented LVEF<40% (i.e. reverse remodelled HFrEF)
- Significant valvular disease (at least moderate or more aortic regurgitation/stenosis, mitral stenosis or severe mitral regurgitation)
- History of myocardial infarction, unstable angina or cerebrovascular accident in the last 6 months
- Symptomatic orthostatic hypotension
- Unfavourable renal anatomy for denervation on renal magnetic resonance imaging (MRI)
- Type 1 diabetes
- Estimated glomerular filtration rate <45ml/min/1.73m² (based on Modification of Diet in Renal Disease criteria)
- On treatment with clonidine, moxonidine, fludrocortisone
- Contraindication to CMR
- Pregnancy

Study Procedures

Study Assessments	Baseline	Randomisation	3 months	12 months
Clinical assessment including MLWHQ	X	Only patients randomised to active treatment will undergo an invasive procedure	X	X
Blood tests	X		X	X
Urine tests	X		X	X
24hr ECG Holter	X		X	X
24hr ambulatory BP	X		X	X
Echocardiogram	X		X	X
MRI- cardiac	X		X	X
MRI- renal	X			X
Cardio-pulmonary exercise test	X		X	X
Cardiac mIBG	X			X
Renal artery ablation		X		

Table S1: Flowchart summarising study procedures. MLWHFQ- Minnesota Living with Heart Failure Questionnaire; MRI- magnetic resonance imaging; mIBG- meta-iodo-benzyl-guanidine

Blood tests

Blood was taken in all patients at the same time on each assessment day. BNP was measured by immunoassay on the Beckman Access 2 Immunoassay analyser (Beckman Coulter, UK).

24 hour ECG Holter

A SEER 12 (GE) digital Holter recorder was used to record a 24hr ECG with a 1024 Hz sample rate. The data was then uploaded on to a MARS ambulatory ECG system for extraction of mean heart rate.

24hr Ambulatory blood pressure monitor (ABPM)

A Spacelabs 90207 ABPM was attached for a period of 24 hours and was analysed using automated and proprietary software.

Echocardiogram

A Philips iE33 machine with a S5-1 transducer was used. LV dimensions were acquired in the parasternal long axis views. LV EF was calculated from biplane echocardiography using Simpson's method. Left atrial volume was estimated using the area-length method from the apical 2 and 4 chamber view. LV diastolic function was assessed using pulse-wave Doppler with the sample volume placed at the mitral valve tip. Tissue Doppler imaging with pulse wave was used to obtain septal and lateral mitral annular velocities (e'). The tissue Doppler images were analysed anonymised by one reader.

Cardiovascular Magnetic Resonance (CMR)

Images were acquired using a Siemens Avanto (1.5 Tesla) scanner. ECG gating was performed throughout. Similar settings were replicated for follow up scans for each patient. After initial localiser and pilot scans were performed, long axis cines through the vertical long axis (two chamber), horizontal long axis (four chamber), left ventricular outflow and right ventricular outflow tract were obtained. Steady-state free precession sequences were

used for the assessment of left (LV) and right (RV) ventricular volumes, left atrial volumes and LV mass. The slice thicknesses were 7mm with a 3mm interval. Typical parameters were as follows: voxel size 2.1 x 2.1 x 7mm, 25 phases were acquired, TR 39ms, TE 1.1ms and flip angle 71°. Biventricular volumes, mass and function were calculated by a single blinded observer (GS) using a semi-automated software (CMRtools, CVIS, UK) using summation of short axis slices. Initially, the LV endocardial and epicardial borders were delineated in all cardiac phases. The systolic descent of the mitral valve was subsequently calculated from tracking of the valve motion in the long axis cines and used to correct for loss of systolic LV volume due to AV ring descent. Finally, the blood pool thresholding was used to delineate the papillary muscles which were carefully excluded from the blood pool and considered part of the left ventricular mass. Atrial volumes were also calculated by summation of a stack of short axis slices by a single blinded observer (VV). All atrial parameters were measured in the ventricular end-systolic phase of the corresponding cine sequences to enable measurement of maximum LA volume.

Renal Magnetic Resonance Angiography (MRA)

Images were acquired using the Siemens Skyra (3.0 Tesla) scanner. After initial scouts and localisers, renal arteries were imaged using a 3D FLASH sequence (voxel size 1mm x 1mm x 1mm; slice thickness 1mm; TR 3.84ms; TE 1.28ms; flip angle 25°) in the coronal plane using an automated dual headed injector to deliver gadobutrol (Gadovist, Bayer, Germany) at a dose of 0.1ml/Kg followed by a saline flush.

Cardiopulmonary exercise testing

All tests were performed using a Medgraphics Ultima CPX (Medical Graphics Corporation, Minnesota, USA) metabolic cart. The machine was calibrated before each study. Exercise

was performed on a treadmill to symptom-limited maximum using a modified Bruce protocol. For those patients in whom a modified Bruce protocol was not tolerated, after a 5 minute rest period, a slower-onset protocol was used. The details of protocol used were recorded and reproduced at each assessment. Peak VO₂ was calculated as the highest O₂ uptake average over the last 1 minute of exercise.

Meta-iodo-benzyl-guanidine (mIBG) scintigraphy

Standardised techniques were used. On the day of the test patients received 400mg of potassium perchlorate (thyroid protection) at least 30 minutes before injection of the radiotracer, ¹²³I- metaiodobenzylguanidine (mIBG) using 200 MBq if BMI≤35 or 400 MBq if BMI>35. The same activity was used for baseline and follow-up scans.

A GE Infinia Hawkeye scanner was used to acquire anterior and posterior planar images (128x128 matrix) of the mediastinum and abdomen 15 minutes and 4 hours after injection.

A heart/mediastinum ratio was calculated from the mean pixel count in a region of interest (ROI) over the heart, including the LV cavity, and the upper mediastinum avoiding the thyroid gland in the late anterior image. Myocardial washout rate (WR) was calculated as:

$$WR = \frac{(H_e - M_e) - [(H_l - M_l) \times 1.217]}{(H_e - M_e)} \times 100$$

, where H= mean pixel count from ROI of heart; M= mean pixel count from ROI of mediastinum; e= early; l= late; 1.217 is the decay factor correction for the late image.

Right heart catheterisation

Prior to performing denervation and receiving any intravenous medications, patients underwent a right heart catheterisation using a 7Fr balloon tipped catheter and in a fasting state. Lidocaine local anaesthetic was used to achieve femoral vein access. No intravenous analgesia or sedatives were given. 5000 units of heparin were administered. The system was 'zeroed' to atmospheric pressure at the level of the heart before any recordings. A 7Fr thermodilution balloon-tipped catheter was advanced to the right atrium, right ventricle, pulmonary artery and wedge position. Blood pressures traces were recorded in each position at end-expiration. A blood saturation >95% was used to confirm an adequate wedge position. Cardiac output (Litres/min) was calculated using the dilution (multiple measurements were taken until there was less than 10% variability in readings) and indirect Fick's method (using blood oxygen content from samples from the pulmonary artery and aorta) as an internal check. A left ventricular end diastolic pressure was obtained using a 6 Fr pigtail catheter retrogradely advanced into the left ventricle via the femoral artery. Trans-pulmonary gradient (TPG) was calculated as: mean pulmonary artery pressure (mPAP) – mean pulmonary capillary wedge pressure (mPCWP). Pulmonary artery resistance (dynes/sec/cm⁵) was calculated as: TPG/cardiac output x 80.

Renal denervation

All procedures were performed by one of two operators with two years prior experience of performing RD. Intravenous opioid, midazolam and/or paracetamol was used to maintain patient comfort during the procedure. Intra-arterial heparin was used to maintain an activated clotting time (ACT) >250 seconds. Access to the renal artery was obtained via the femoral artery using an 80 cm 6 Fr guide catheter (RDC or IMA curve). The Symlicity™ catheter was then advanced and positioned in the most distal aspect of the renal artery proximal to bifurcations, good vessel wall contact was obtained by deflecting the electrode tip and

confirmed by an impedance of 250-300 Ohms on the power generator. The interventionists sought to apply a minimum of four ablations to each main renal artery in a circumferential distribution.

Results

Patient ID	Mean PAP (mmHg)	Mean PCWP (mmHg)	LVEDP (mmHg)	TPG (mmHg)	PVR (dynes/sec/cm ⁵)	MABP (mmHg)
2	24	16	22	8	149	68
4	20	15	17	5	52	109
5	24	13	21	11	121	82
6	28	16	21	12	168	90
8	20	17	15	3	39	66
9	26	24	13	2	25*	116
10	20	11	13	9	149*	94
11	24	19	15	5	60	105
13	26		17	9	150	101
15	17	14	15	3	45	76
16	43	21	19	22	201	97
17	46	39	22	7	126	92
19	43	25	27	18	436	129
21	31		22	9	124	90
23	28	16	17	12	126	105
24	25	22	17	3	46	74
25	22	17	19	5	85	88
No. Elevated	9 (53%)	12 (80%)	12 (71%)	3 (18%)	1 (6%)	5 (29%)

Table S2: Right and left heart catheterisation haemodynamic data for the 17 patients

who received renal denervation. PAP- pulmonary artery pressure; PCWP- pulmonary capillary wedge pressure; LVEDP- left ventricular end-diastolic pressure; MABP- mean arterial blood pressure; TPG- trans-pulmonary gradient; pulmonary vascular resistance. *

Derived using the indirect Fick's cardiac output to rather than thermodilution cardiac output.

Highlighted in pink are abnormally elevated parameters.

	RD			Control			Change from baseline							
	Baseline (n=17)	3 Months (n=16)	12 Months (n=17)	Baseline (n=8)	3 Months (n=8)	12 Months (n=7)	3 Months				12 Months			
							RD	Control	P	P*	RD	Control	P	P*
MLWHFQ	48 (27-56)	38 (30-46)	35 (27-50)	28 (25-50)	35 (16-42)	25 (14-36)	-9 (-15, 10)	-6 (-12, 6)	0.988	0.699	-7 (-20, 4)	-7 (-10, 0)	0.721	0.708
VO₂ Peak (ml/kg/min)	14.7 ± 3.3	15.9 ± 4.1	14.0 ± 3.2	17.5 ± 3.8	16.8 ± 5.4	18.1 ± 5.3	+1.4 ± 1.9	-0.7 ± 4.4	0.112	0.150	+0.2 ± 2.0	+0.8 ± 4.3	0.637	0.472
BNP (ng/L)	210 (137-354)	234 (133-327)	215 (142-351)	149 (99-205)	183 (90-283)	125 (112-262)	-19 (-59, 54)	+28 (-26, 124)	0.257	0.763	-3 (-59, 33)	+18 (-2, 30)	0.318	0.559
E/e' (average)	14.6 ± 6.7	12.3 ± 4.5	14.0 ± 6.6	14.4 ± 3.9	16.7 ± 2.3	13.9 ± 3.4	-1.1 ± 5.7	+2.3 ± 3.1	0.123	0.015	+0.2 ± 4.4	+0.2 ± 1.2	0.974	0.962
Left atrial volume (ml/m²)	62.8 ± 29.2	64.9 ± 30.1	69.3 ± 32.5	61.3 ± 21.7	65.1 ± 26.7	60.7 ± 36.1	+1.3 ± 8.8	-1.3 ± 8.2	0.495	0.518	+6.5 ± 13.2	+2.4 ± 8.9	0.461	0.504
Left ventricular mass (g/m²)	62.9 ± 11.4	62.9 ± 11.8	63.5 ± 12.4	64.6 ± 7.1	63.4 ± 6.0	65.3 ± 7.2	+0.4 ± 2.8	-1.1 ± 2.7	0.214	0.250	+0.7 ± 4.3	+0.2 ± 3.2	0.803	0.807

Table S3: Primary efficacy endpoints at baseline, 3 months and 12 months. Data are presented as mean ± standard deviation or median

(quartile 1, quartile 3). P- represents unadjusted independent samples t-test or Mann-Whitney U test. P* -values are adjusted for baseline values

using an ANCOVA model.

Patient ID	No. Right Renal Artery Ablations				No. Left Renal Artery Ablations			
	Sup	Inf	Lat	Total	Sup	Inf	Lat	Total
2	0	3	1	4	3	0	1	4
4	2	1	1	4	2	2	1	5
5	2	2	1	5	3	1	1	5
6	1	2	1	4	2	2	2	6
8	1	1	2	4	2	1	2	5
9	3	1	2	6	0	0	0	0
10	2	1	1	4	3	1	2	6
11	0	2	2	4	2	2	2	6
13	2	2	2	6	2	1	2	5
15	2	1	2	5	3	2	1	6
16	2	1	3	6	1	1	2	4
17	2	3	1	6	2	2	1	5
19	3	2	1	6	2	2	3	7
21	1	1	2	4	2	1	2	5
23	2	3	2	7	2	3	1	6
24	1	2	2	5	1	3	1	5
25	1	3	1	5	2	1	1	4
Median (IQR)	2 (1, 2)	2 (1, 2)	1 (1, 2)	5 (4, 6)	2 (2, 2)	1 (1, 2)	1 (1, 2)	5 (5, 6)

Table S4: Number and pattern of radiofrequency energy applications in the RDT-PEF

trial. Highlighted (pink) patients are those who had anatomically incomplete ablations. Sup-

superior; Inf- inferior; Lat- lateral; IQR- interquartile range.

Endpoints	Change at 3 months from Baseline			Change at 12 months from Baseline		
	Complete Ablation (n=5)	Incomplete Ablation (n=12)	P	Complete Ablation (n=5)	Incomplete Ablation (n=12)	P
Efficacy						
MLWHFQ	+8 (-27, 20)	-10 (-15, 4)	0.446	-20 (-22, 1.5)	-4 (-13, 5)	0.234
VO₂ Peak (ml/kg/min)	+2.6 ± 1.5	+1.0 ± 1.9	0.262	1.3 ± 1.9	-0.2 ± 4.6	0.343
BNP (ng/L)	+37 (-9, 54)	-33 (-63, 66)	0.212	+20 (-47, 49)	-21 (-81, 13)	0.383
E/e' (average)	-5.2 ± 7.4	+0.2 ± 4.6	0.133	-0.3 ± 6.9	+0.4 ± 3.2	0.646
Left atrial volume (ml/m²)	+1.8 ± 8.2	+1.2 ± 9.3	0.862	+10.5 ± 13.3	+4.9 ± 13.4	0.506
Left ventricular mass (g/m²)	+1.8 ± 2.9	-0.1 ± 2.8	0.316	+1.6 ± 2.5	+0.3 ± 4.9	0.721
Safety						
eGFR (ml/min/1.73m²)	-6 (-7, -4)	-3 (-11, 3)	0.599	-2 (-12, 5)	-3, (-11, 3)	0.879
Autonomic Assessments						
Plasma NA (nmol/L)	-0.62 ± 3.41	+0.19 ± 1.93	0.541	+0.27 ± 1.95	-0.16 ± 1.78	0.664
24hr Urine NA (nmol/24hr)				-4.8 ± 22.7	-26.9 ± 100.5	0.484
Early HMR				-0.04 (-0.14, 0.03)	+0.05 (-0.12, 0.25)	0.383
Late HMR				-0.07 ± 0.12	+0.03 ± 0.10	0.099
Washout rate (%)				+0.69 ± 21.05	-5.67 ± 18.91	0.549

Table S5: Subgroup analyses comparing change in efficacy endpoint, estimated glomerular filtration rate (eGFR) and autonomic assessments between those patients that received anatomically completed ablation and those with incomplete. NA- noradrenaline; HMR- heart: mediastinum form mIBG imaging. Data are presented as mean ± standard deviation or median (quartile 1, quartile 3). p represents Mann-Whitney U test.

Medication Doses	Three Months		Twelve Months	
	Altered	No Change	Altered	No Change
RD Patients	8	8	11	6
Control Patients	3	5	4	3
P	0.679		1.000	

Table S6: Number of patients who had medication alterations during the course of the RDT-PEF trial. P value derived from the chi-squared test

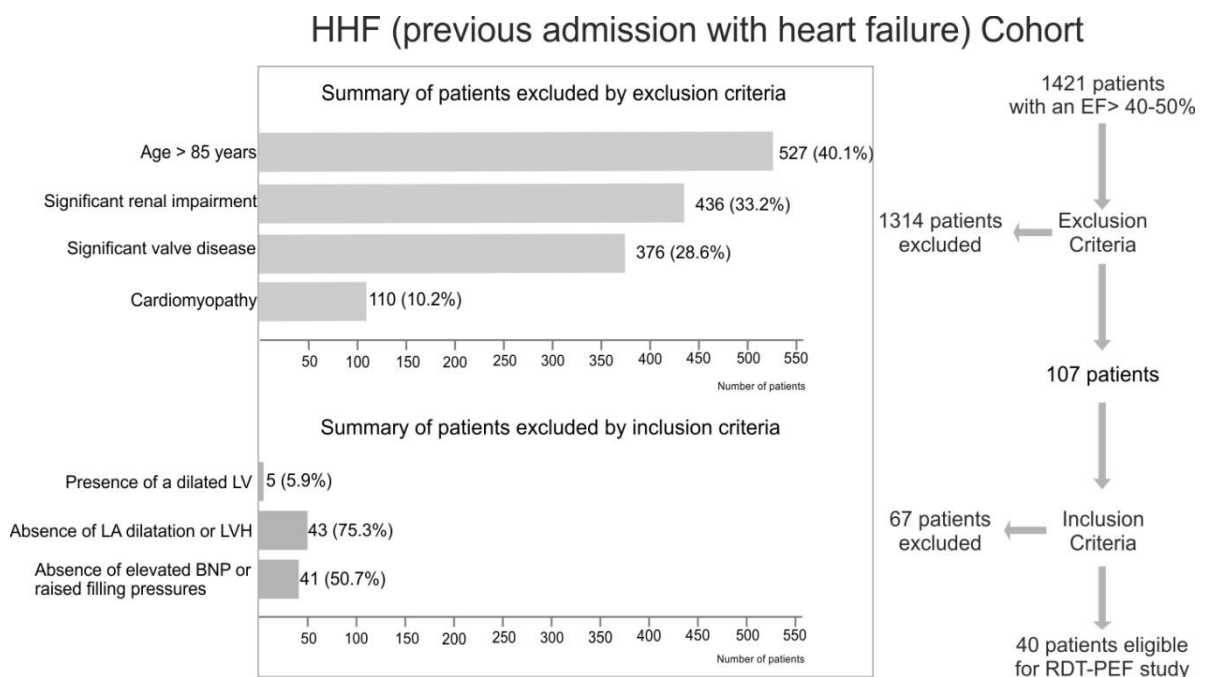


Figure S7: Bar chart illustrating underlying reasons for screen failure in those patients with an EF > 40-50% from the HHF (patients with a previous admission for heart failure) cohort only. LV- left ventricle, LA- left atrial; LVH- left ventricular hypertrophy; BNP- B-type natriuretic peptide.