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Repeatability and sensitivity to change of non-invasive end points in PAH: the RESPIRE study

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Abstract End points that are repeatable and sensitive to change are important in pulmonary arterial hypertension (PAH) for clinical practice and trials of new therapies. In 42 patients with PAH, test–retest repeatability was assessed using the intraclass correlation coefficient and treatment effect size using Cohen's d statistic. Intraclass correlation coefficients demonstrated excellent repeatability for MRI, 6 min walk test and log to base 10 N-terminal pro-brain natriuretic peptide (log_{10}NT-proBNP). The treatment effect size for MRI-derived right ventricular ejection fraction was large (Cohen's d 0.81), whereas the effect size for the 6 min walk test (Cohen's d 0.22) and log_{10}NT-proBNP (Cohen's d 0.20) were fair. This study supports further evaluation of MRI as a non-invasive end point for clinical assessment and PAH therapy trials.
Trial registration number NCT03841344.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is progressive, leading to right ventricular (RV) failure and death.[1] Accurate measurement of RV function is important for assessment of disease severity and prognosis.[2–4] Despite new therapies and improvements in survival,[5] PAH remains a life-shortening condition. MRI is the gold standard for RV assessment,[6] has prognostic value[2] and predicts clinical worsening[7] in PAH. A trial end point that is highly repeatable, is sensitive to treatment and predicts outcomes would be highly desirable.[8 9] MRI has been proposed as a trial end point in PAH,[8 9] however, there is limited data on repeatability and treatment effect size.

METHODS

Patients

Patients with PAH who were treatment-naïve commencing therapy, prevalent undergoing escalation of therapy and clinically stable requiring no escalation of therapy, were recruited. See online supplemental file S1.

Study investigations

Investigations performed at visit 1 included N-terminal pro-brain natriuretic peptide (NT-ProBNP), 6 min walk test (6MWT) and MRI. Follow-up visits 2 and 3 occurred approximately 6 months after study visit 1. Visits 2 and 3 occurred within 24 hours of each other (online supplemental figure S2).

MRI acquisition and analysis

All MRI examinations were performed on either a 1.5 T GE HDx (GE Healthcare, Milwaukee, USA) whole body scanner using an 8-channel cardiac coil or a 3 T Philips Ingenia (Best, The Netherlands) whole body scanner using a 32-channel dStream torso coil (online supplemental file S1). Analysis of MRI was undertaken blinded to the patient's data. RV parameters and pulmonary arterial flow were analysed on Qmass MEDIS suite (V.3.0.18.0, Medical Imaging Systems, The Netherlands) on short axis and phase contrast images, respectively. Regions of interest were drawn on the pulmonary artery and left atrium of the dynamic contrast-enhanced perfusion images to calculate first pass pulmonary transit time and full width at half maximum using in-house software (see online supplemental figure S3).

Six min walk test and NT-ProBNP

The 6MWT was performed by a respiratory physiologist. NT-ProBNP analysis was performed on patient plasma samples using the Luminex 100/200 multiplex analyser using the cardiovascular marker kit (HCVD-1MAG-67K Millipore) at the end of the study.

Statistical analysis

Repeatability was determined by the intraclass correlation coefficient (ICC) using a two-way mixed absolute agreement model with the average measure recorded. An ICC of ≥0.75 was considered excellent, 0.60–0.74 good, 0.40–0.59 fair and <0.40 poor. Mean difference and 95% CIs were presented where appropriate. Cohen's d (calculated with the averaged SD, d_{av}) was used to assess the standardised treatment effect size between visit 1 and visit 2.[10] A Cohen's d value of <0.20 was considered no change, 0.20–0.49 was considered fair change, 0.50–0.79 was considered a medium change and ≥0.80 was considered a large change. All analysis was performed on SPSS V.22 and GraphPad Prism V.16.

RESULTS

Patients

Of 42 patients who completed the study, 16 were incident and treatment-naïve and initiated PAH therapy, 12 were prevalent and underwent an escalation of therapy and 14 were stable on therapy with no change in treatment occurring between the study visits.(online supplemental table S5).

Table 1 Repeatability in all patients with PAH (ICC), and treatment effect size for patients with PAH initiating or escalating PAH therapy

	All PAH		Patients with PAH initiating or escalating therapy										
				Visit 1		Visit 2		Change (Visit 1–visit 2)			95% CI		
	N	ICC	N	Mean	SD	Mean	SD	Mean difference	SD	SEM	Lower	Upper	Cohen's d
Walk test													
6MWT distance (m)	39	0.987	24	325.63	156.30	361.50	166.29	−35.88	79.06	16.14	−69.26	−2.49	0.22
Blood tests													
Log NT-ProBNP	32	0.772	24	2.76	0.46	2.67	0.41	0.09	0.32	0.07	−0.05	0.22	0.20
MRI metrics													
SA with threshold													
RVEDM (g)	40	0.970	26	117.80	45.72	99.40	43.96	18.40	30.90	6.06	5.92	30.88	0.41
RVESM (g)	40	0.980	26	106.68	39.73	94.61	42.08	12.06	26.79	5.25	1.24	22.88	0.29
RVEDV (mL)	40	0.969	26	145.71	39.12	146.03	55.87	−0.32	29.13	5.71	−12.08	11.45	0.01
RVESV (mL)	40	0.983	26	93.93	34.66	81.28	41.40	12.65	22.02	4.32	3.76	21.55	0.33
RVEF (%)	40	0.883	26	36.56	11.48	45.69	11.12	−9.12	10.45	2.05	−13.35	−4.90	0.81
RVSV (mL)	40	0.864	26	51.78	17.30	64.75	23.92	−12.97	23.27	4.56	−22.37	−3.57	0.62
RVCO (L/min)	40	0.886	26	3.95	1.45	4.48	1.55	−0.53	1.54	0.30	−1.15	0.09	0.35
Systolic septal angle (°)	40	0.852	27	163.33	16.45	156.81	14.00	6.52	11.28	2.17	2.06	10.98	0.43
Diastolic septal angle (°)	40	0.897	27	153.11	14.73	145.48	10.44	7.63	10.15	1.95	3.61	11.65	0.60
Q flow													
Net flow volume (mL)	41	0.893	26	58.05	30.18	69.49	31.30	−11.44	34.83	6.83	−25.51	2.62	0.37
Forward flow volume (mL)	41	0.860	26	60.37	27.58	72.33	29.15	−11.96	31.97	6.27	−24.88	0.95	0.42
Backward flow volume (mL)	41	0.817	26	2.32	6.76	2.84	5.74	−0.52	5.85	1.15	−2.88	1.85	0.08
Regurgitant fraction (%)	41	0.731	26	6.28	19.58	5.42	11.52	0.87	18.77	3.68	−6.71	8.45	0.05
Average flow velocity (cm/s)	41	0.909	26	7.31	3.60	8.25	3.69	−0.94	3.69	0.72	−2.43	0.55	0.26
Peak flow velocity (cm/s)	41	0.582	26	52.97	16.37	67.68	22.71	−14.71	19.35	3.79	−22.53	−6.90	0.74
Diastolic vessel area (mm^2)	41	0.933	26	981.10	257.92	961.84	242.96	19.26	104.52	20.5	−22.96	61.48	0.08
Systolic vessel area (mm^2)	41	0.953	26	1077.57	279.96	1083.62	266.78	−6.05	101.08	19.82	−46.88	34.77	0.02
Pulmonary arterial pulsatility (%)	41	0.776	26	9.96	4.87	13.00	5.12	−3.04	3.62	0.71	−4.50	−1.58	0.61
DCE imaging													
Pulmonary transit time (s)	36	0.728	21	6.76	1.81	6.12	1.88	0.64	1.60	0.35	−0.09	1.37	0.35
FWHM (s)	32	0.906	18	7.89	3.14	6.20	2.40	1.68	2.19	0.52	0.60	2.77	0.60

Data are shown for all patients with PAH initiating or escalating PAH therapy.
DCE, dynamic contrast-enhanced imaging; FWHM, full width at half maximum; ICC, intraclass correlation coefficient; Log_{10}NT-ProBNP, log to base 10 N-terminal pro-brain natriuretic peptide; 6MWT, six min walk test; PAH, pulmonary arterial hypertension; RVCO, right ventricular cardiac output; RVEDM, right ventricle end-diastolic mass; RVEDV, right ventricle end-systolic volume; RVEF, right ventricle end-systolic volume; RVESM, right ventricle end-systolic mass; RVSV, right ventricle stroke volume; SA, short axis.

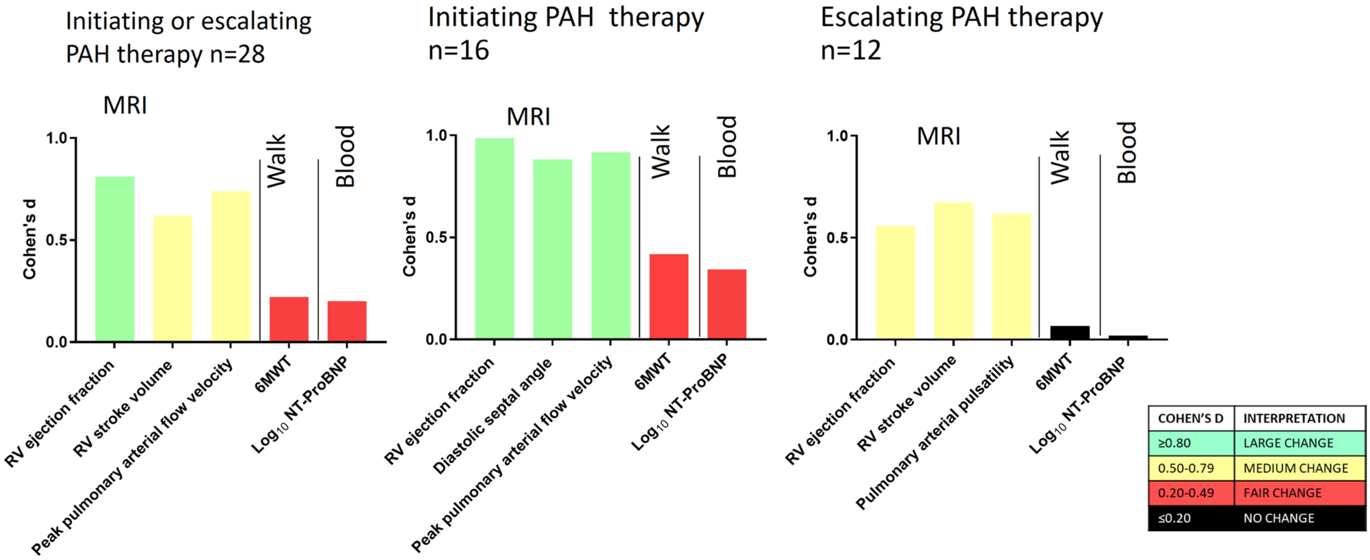

Figure 1 Comparison of treatment effect size using Cohen's d results in patients initiating and/or escalating pulmonary arterial hypertension (PAH) therapy. 6MWT, 6 min walk test; Log_{10}NT-ProBNP, log to base 10 N-terminal pro-brain natriuretic peptide; RV, right ventricular.

Test–test repeatability (visits 2 and 3)

In patients with PAH, test–test repeatability was assessed between visits 2 and 3; 6MWT (ICC 0.987) and log_{10}NT-ProBNP (ICC 0.772) had excellent repeatability. Of cardiac MRI metrics (table 1), all showed excellent repeatability. Data for MRI pulmonary flow and perfusion transit times are shown in table 1.

Treatment effect size (visits 1 and 2)

For all patients, initiating or escalating therapy (n=28), the only measurement with a large treatment effect size was RV ejection fraction (Cohen's d 0.81). The 6MWT (Cohen's d 0.22) and NT-ProBNP (Cohen's d 0.20) demonstrated a fair treatment effect size (table 1). Figure 1 shows Cohen's d values for the top three MRI end points, the 6MWT and NT-proBNP. Figure 2 shows ICC versus Cohen's d value for all end points. In patients initiating PAH therapy, RV ejection fraction (Cohen's d 0.99), diastolic septal angle (Cohen's d 0.88) and peak pulmonary arterial flow velocity (Cohen's d 0.92) had a large treatment effect size. In patients escalating therapy, RV ejection fraction, RV stroke volume and pulmonary arterial pulsatility had a medium effect size, whereas NT-ProBNP (Cohen's d 0.02) and 6MWT (Cohen's d 0.07) demonstrated no treatment effect (see online supplemental figure S4). The stable patient group showed either no or fair changes across all measured parameters (online supplemental table S6).

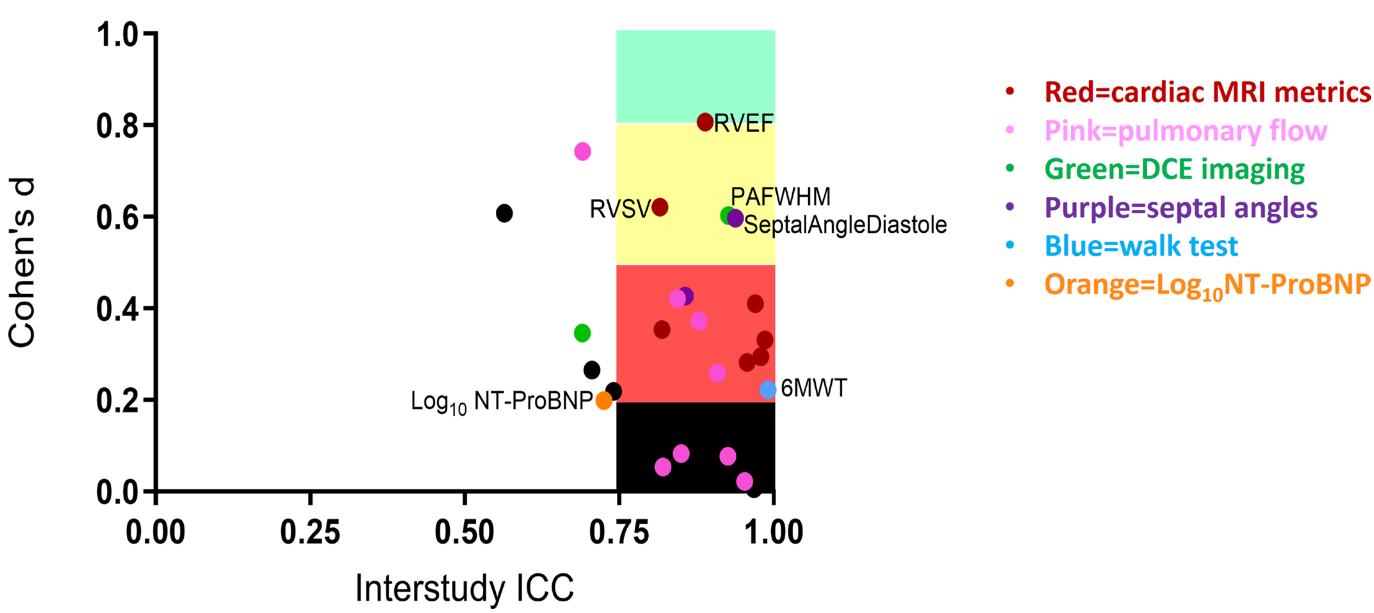

Figure 2 Cohen's d versus interstudy intraclass correlation coefficient (ICC) for study measurements. DCE, dynamic contrast-enhanced imaging; Log_{10}NT-ProBNP, log to base 10 N-terminal pro-brain natriuretic peptide; PAFWHM, pulmonary arterial full width at half maximum; RVEF, right ventricular ejection fraction; RVSV, right ventricle stroke volume; 6MWT 6 min walk test. ICC >0.75=excellent repeatability. Cohen's d value of <0.20 was considered no change, 0.20–0.49 was considered fair change, 0.50–0.79 was considered a medium change and ≥0.80 was considered a large change.

DISCUSSION

Investigations used to monitor disease severity in patients with PAH, namely 6MWT distance, NT-ProBNP level and MRI metrics, had excellent repeatability. In contrast, only MRI (RVEF) demonstrated a large treatment effect size in patients initiating or escalating therapies, whereas for the 6MWT and NT-ProBNP the treatment effect sizes were fair.

As observed in previous clinical trials[1] and highlighted at the 6th World Symposium,[9] all metrics evaluated in patients with PAH escalating therapy had a lower treatment effect size compared with treatment-naïve patients initiating therapy. This represents a challenge when studying the effects of new therapies in PAH where the standard of care is combination treatment.[1] Importantly, MRI was still able to detect a medium treatment effect size in patients receiving background PAH therapy. Due to the large cost of conducting PAH therapy trials, strategies to reduce the size of studies and their duration using a surrogate end point that is repeatable and has a large treatment effect size would be highly desirable.[9]

This study has a number of limitations including the small sample size and the lack of comparison with invasively measured pulmonary haemodynamics. Nonetheless, we have demonstrated in this exploratory study that MRI, the gold standard for RV function assessment, detects a larger treatment effect than the 6MWT or NT-proBNP. This may reflect the ceiling effect of the 6MWT and the effect of comorbidities (including chronic kidney disease) that may influence 6MWT distance and NT-proBNP levels. MRI metrics predict clinical worsening[7] and mortality[2–4] fulfilling many of the criteria of a surrogate end point.[9] Given that pulmonary haemodynamics are commonly used in early phase PAH studies,[1] a direct comparison of MRI metrics and pulmonary haemodynamics, to detect longitudinal change following PAH therapy, is now required if MRI imaging is to be considered a primary end-point for PAH therapy trials.[8,9]

This study demonstrates the high repeatability of MRI metrics in PAH and the large treatment effect size support further evaluation of MRI as a non-invasive endpoint in PAH therapy trials.

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Contributors AJS, FW, JMW, AC, LK, DGK conceived the idea for the study. MC, MA, DGK, RC, AJS supported patient recruitment. AJS, JMW, DGK, AM, PH, LS devised the MRI protocol. AJC, CO, PH, LS analysed the MRI studies. FA, AM, CJ, PH, PG performed data quality control checks. MA performed the walk tests and FA, JP, AL performed the lab analyses. MC, LK, SA, AR, PG, AJS, YS, FS, PH, LS supported the data collation and analysis. Statistical analysis was performed by MC, SA, AJS, FA, LS, LK. All authors contributed to the drafting of the manuscript. All authors approved the final version of the manuscript.

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Competing interests FW, LK and AC are employees and shareholders of GlaxoSmithKline. AS is the principal investigator for the collaborative research grant from GlaxoSmithKline that funded this study. AS has undertaken consultancy work for General Electric and Actelion Pharmaceuticals. RC has received fees for lecturing and participation in advisory boards, from Actelion, Bayer, GSK and MSD. DGK has received fees for lecturing and participation in advisory boards, from Actelion, Bayer, GSK and MSD and fees for participation in Steering Committees for Actelion.

Patient consent for publication Not required.

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Supplementary file S1

Methods

Patients

Patients with PAH were prospectively recruited from a nationally designated pulmonary hypertension referral centre. We aimed to recruit 3 approximately equal size groups of patients: incident treatment-naïve patients commencing therapy, prevalent patients escalating therapy and patients who were deemed to be clinically stable and required no escalation of therapy. For study inclusion, patients were required to have idiopathic pulmonary arterial hypertension (IPAH), heritable PAH, PAH associated with connective tissue disease or portal hypertension, to have undergone RHC and have a mean pulmonary arterial pressure ≥ 25 mmHg and pulmonary arterial wedge pressure ≤ 15 mmHg and to have had other causes of pulmonary hypertension excluded. Exclusion criteria included pregnancy, allergy to contrast medium, contraindication to MRI or known active Hepatitis B or C or HIV infection. Ethical and institutional review board approval was obtained and all patients provided informed written consent.

Study investigations

Investigations performed at visit 1 included a blood draw (NT-ProBNP), an exercise test (6MWT) and MRI. Any subsequent treatment initiation or escalation occurred after visit 1 investigations were completed. Follow-up visits 2 and 3 occurred approximately 6 months after study visit 1. Visits 2 and 3 occurred within 24 hours of each other. Typically, visit 2 investigations occurred in the morning, and visit 3 investigations occurred in the afternoon or the following day. The study flow diagram, **Supplementary figure S2**, describes the study investigations. Patients took their usual medications approximately 1 hour prior to their MRI. Patients rested for 45 minutes after the 6-minute walking test to allow for recovery as per ATS recommendations. Patients were asked to refrain from exercise, caffeinated drinks and alcohol between investigations during their visits. Follow up scans at visits 2 and 3 were performed on the same MRI system as at visit 1.

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MRI acquisition

The rationale for performing measurements on two different MRI scanners at different field strengths and from different vendors was to assess the variation introduced in the candidate MRI measurements across platforms. Short axis cine cardiac imaging stacks were performed using a balanced single shot free precession sequence with full ventricular coverage. End-systole was defined as the smallest chamber area on each slice and end-diastole was defined as the first cine phase of the R-wave triggered acquisition. The phase-contrast velocity imaging was performed 1cm above and orthogonal to the pulmonary valve. For dynamic contrast enhanced perfusion imaging a three-dimensional spoiled gradient echo time-resolved Magnetic Resonance Angiography sequence was used, positioned coronal across the chest with full lung coverage with an intravenous bolus injection of Gadovist (Bayer Healthcare, Germany) (0.05 mL/kg at 4ml/s followed by a saline flush of 20mL). Sequence parameters were: TR=2.1ms, T=0.7ms, FA=30°, BW=250kHz, with a reconstructed voxel size of 1.875x1.875x10mm and an effective frame rate of ~0.5s per whole lung volume.

MRI analysis

Epicardial and endocardial contours were drawn using semi-automatic tools with intensity thresholding of the structures within the endocardial contour, and biventricular volume, mass and function recorded. Regions of interest were drawn on the pulmonary artery and left atrium of the dynamic contrast enhanced perfusion images to calculate first pass pulmonary transit time (PTT) and full-width-half-maximum (FWHM) using in-house software. See **Supplementary figure S3**

Statistical analysis

Following histogram analysis, variables were categorised into normal and non-normal distributions: if normally distributed, mean and standard deviation were presented, whereas if non-normally distributed, median and interquartile range were presented.

Results

Patients

A total of 104 patients with PAH or suspected PAH were approached to take part in the study, 58 patients with suspected PAH were consented; of the 58 patients, 44 met the entry criteria after completion of their baseline study investigations and 42 completed the study. Patients had a mean age of 51 years and 83% were female. **Supplementary Table S5** presents demographic, pulmonary function test and right heart catheter data for all patients. Two patients experienced some claustrophobic symptoms at both visits but completed the study protocol. The mean time intervals between baseline and follow up for these 3 subgroups were: 6 months (standard deviation (SD) 3 months), 6 months (SD 1 month), and 8 months (SD 2 months) respectively. MRI and 6MWT were normally distributed on visual inspection of histograms. NT-ProBNP had a skewed distribution, however, following $\log_{10}$ transformation the distribution was normalised.

Supplementary figures

S2 Study flow chart illustrating the three study visits and mandatory investigations. All patients and healthy volunteers included in the study underwent the same protocol

S3 Illustration of the diastolic (A) and systolic (B) short axis cardiac cine MRI (epicardial and endocardial contours drawn and thresholding applied). Magnitude (C) and flow (D) images from phase contrast MRI sequence placed orthogonal to the main pulmonary artery. Dynamic contrast enhanced images illustrating contrast in the pulmonary arteries (E) and passing through to the left atrium, left ventricle and aorta (F).

S4 Line plots showing the changes in right ventricular ejection fraction (RVEF) and right ventricular stroke volume (RVSV) for individual patients in the combined initiating and escalating therapy group.

Supplementary tables

Supplementary table S5: Demographics, therapy, diagnostic and right heart catheter data in patients with PAH.

	All patients n=42	Treatment naïve n=16	Treatment change n=12	Stable n=14
Demographics				
Age (yrs)	51 (15)	54 (14)	52 (16)	46 (15)
Female [n (%)]	35 (83)	14 (88)	10 (83)	11 (79)
WHO functional class	I (0) II (2) III (35) IV (5)	I (0) II (0) III (13) IV (3)	I (0) II (0) III (11) IV (1)	I (0) II (2) III (11) IV (1)
Treatment (visit 1 therapy)				
Monotherapy	10 (24%)	0 (0%)	8 (67%)	2 (14%)
Combination therapy	10 (24%)	0 (0%)	4 (33%)	6 (43%)
Parenteral prostanoid	6 (14%)	0 (0%)	0 (0%)	6 (43%)
Calcium channel blocker	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Treatment (visit 2 therapy)				
Monotherapy	4 (10%)	1 (6%)	1 (8%)	2 (14%)
Combination oral therapy	23 (54%)	9 (56%)	8 (67%)	6 (43%)
Parenteral prostanoid	13 (31%)	4 (25%)	3 (25%)	6 (43%)
Calcium channel blocker	2 (5%)	2 (13%)	0 (0%)	0 (0%)
Subgroups				
IPAH and HPAH	25 (60.9%)	8 (50%)	8 (66.6%)	10 (71.1%)
PAH CTD	10 (24.4%)	6 (37.5%)	2 (16.6%)	2 (14.3%)
PAH congenital	1 (2.4%)	1 (6.25%)	0 (0%)	0 (0%)
PAH portal	4 (10%)	1 (6.25%)	1 (8.3%)	2 (14.3%)
PAH drug and toxins	1 (2.4%)	0 (0%)	1 (8.3%)	0 (0%)
Walk test				
6MWT Distance (m)	375 (167)	303 (144)	332 (179)	488 (120)
Blood tests				
Log_{10}NT-ProBNP*	561 (258 to 906)	619 (213 to 1095)	665 (301 to 1356)	386 (176 to 606)
Right heart catheter				
mRAP (mmHg)	11 (7)	13 (7)	14 (7)	6 (2)
mPAP (mmHg)	52 (10)	53 (7)	58 (10)	46 (14)
PCWP (mmHg)	10 (3)	10 (2)	12 (2)	10 (3)
CI (L/min/m2)	2.4 (0.9)	2.0 (0.8)	2.0 (0.7)	3.0 (0.8)
PVR (Wood units)	11 (6)	14 (6)	13 (5)	7 (5)
Mixed venous oxygen saturations (%)	64 (10)	60 (11)	64 (6)	69 (7)

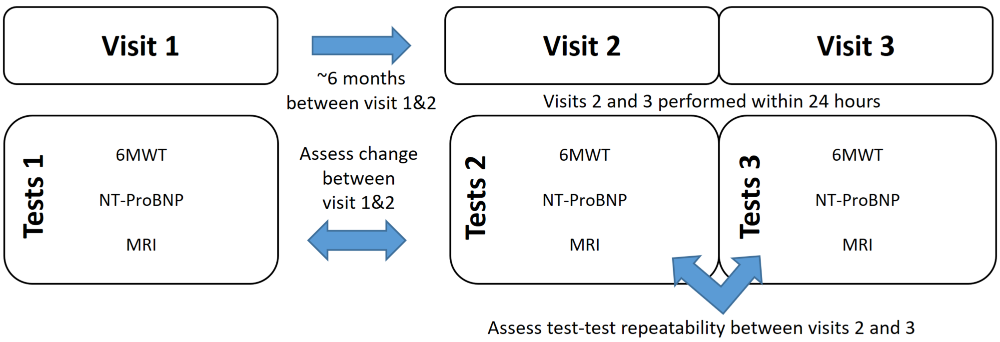
Values are mean (SD) or n (%) unless otherwise stated
* Median (IQR) presented

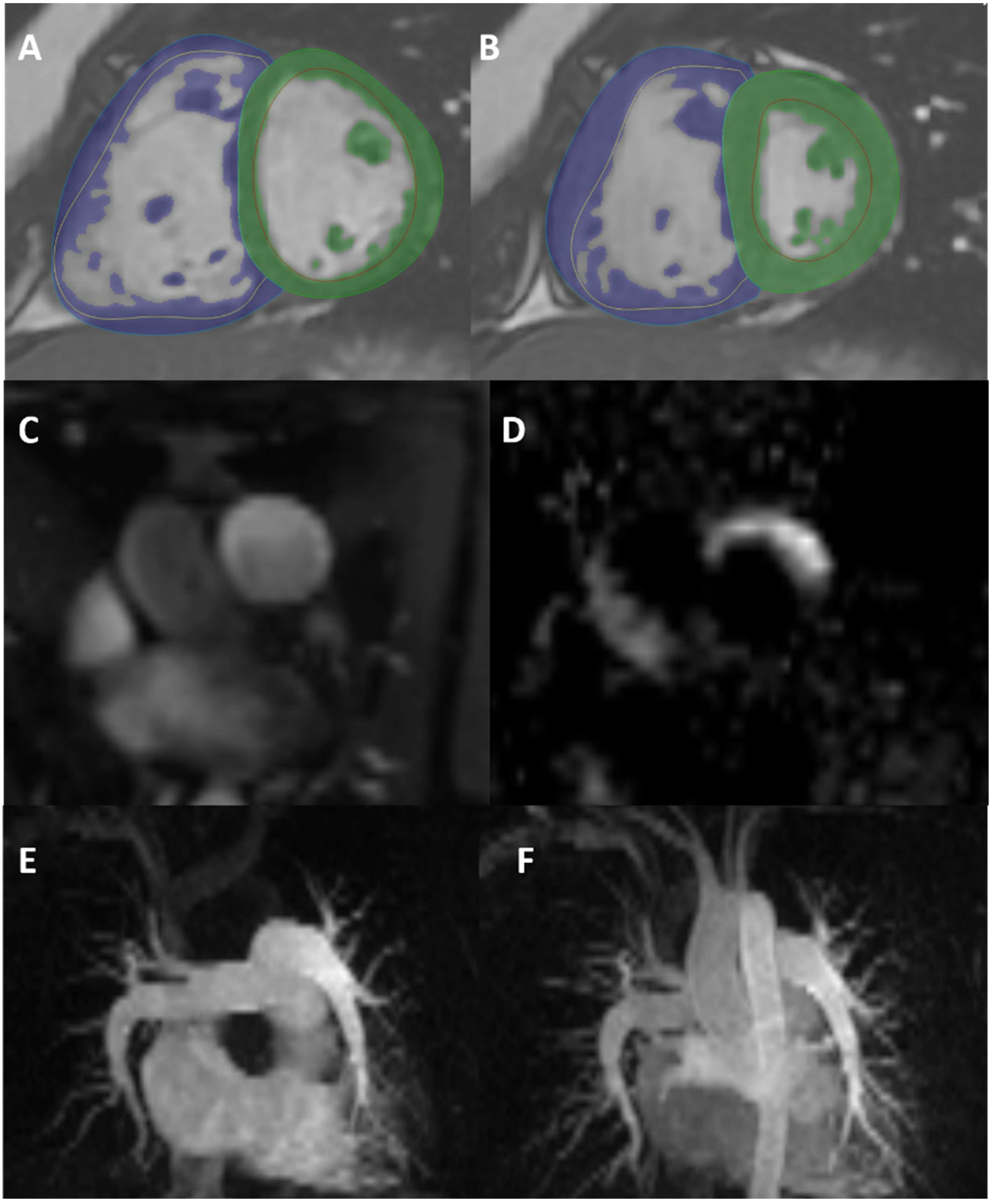
Supplementary table S6 Mean and standard deviation of walk test, NT-ProBNP and MRI metrics in stable patients with PAH who do have no change in PAH therapy between visit 1 or visit 2.

	N	Visit 1		Visit 2		Change (Visit 1 – Visit 2)			95% Confidence Interval		Cohen's d
		Mean	SD	Mean	SD	Mean difference	SD	SEM	Lower	Upper	
Walk test											
6MWT Distance (m)	12	479.83	121.47	473.50	117.98	6.33	80.62	23.27	-44.89	57.56	0.05
Blood test											
Log NT-ProBNP	14	2.53	0.44	2.67	0.32	-0.14	0.37	0.10	-0.35	0.08	0.36
SA with threshold											
RVEDM (g)	14	85.34	27.13	82.55	26.41	2.79	10.47	2.80	-3.25	8.83	0.10
RVESM (g)	14	72.00	24.77	75.33	30.28	-3.33	13.21	3.53	-10.96	4.30	0.12
RVEDV (ml)	14	97.89	31.92	105.75	41.23	-7.86	23.09	6.17	-21.19	5.47	0.21
RVESV (ml)	14	49.18	20.47	52.07	19.88	-2.89	9.26	2.47	-8.24	2.45	0.14
RVEF (%)	14	50.45	8.51	49.58	11.01	0.87	13.54	3.62	-6.95	8.69	0.09
RVSV (ml)	14	48.70	15.29	53.67	26.94	-4.97	25.42	6.79	-19.65	9.71	0.23
RVCO (L/min)	14	3.27	1.12	3.60	2.06	-0.33	1.74	0.47	-1.34	0.68	0.20
Systolic septal angle (°)	13	149.69	16.24	153.00	12.01	-3.31	14.44	4.00	-12.03	5.42	0.23
Diastolic septal angle(°)	13	142.31	10.56	143.15	9.11	-0.85	5.76	1.60	-4.32	2.63	0.09
Q flow											
Net flow volume (ml)	13	79.94	55.65	72.38	32.66	7.56	45.72	12.68	-20.07	35.18	0.17
Forward flow volume (ml)	13	82.17	53.54	74.39	30.50	7.78	43.20	11.98	-18.33	33.89	0.18
Backward flow volume (ml)	13	2.24	5.05	2.02	3.83	0.22	4.85	1.35	-2.71	3.15	0.05
Regurgitant fraction (%)	13	5.67	12.77	4.87	10.24	0.81	12.74	3.53	-6.89	8.51	0.07
Average flow velocity (cm/s)	13	7.97	4.10	8.74	4.57	-0.78	3.00	0.83	-2.59	1.04	0.18
Peak flow velocity (cm/2)	13	66.06	32.37	69.33	29.30	-3.27	39.47	10.95	-27.12	20.58	0.11
Diastolic vessel area (mm2)	13	988.46	314.83	916.21	290.08	72.26	241.04	66.85	-73.40	217.91	0.24
Systolic vessel area (mm2)	13	1110.66	305.79	1042.87	287.10	67.78	245.12	67.98	-80.34	215.91	0.23
Pulmonary arterial pulsatility (%)	13	13.98	6.78	15.25	7.02	-1.27	5.42	1.50	-4.55	2.01	0.18
DCE imaging											
Pulmonary Transit Time (s)	11	5.67	1.13	5.91	1.67	-0.25	1.34	0.40	-1.15	0.65	0.17
FWHM (s)	11	5.21	1.68	5.02	1.50	0.20	1.66	0.50	-0.92	1.31	0.12

6MWT= six minute walk test, Log10NT-ProBNP=log to base 10 N Terminal brain naturitic peptide, SA= short axis, RVEDM=right ventricle end-diastolic mass, RVESM=right ventricle end-systolic mass, RVEDV=right ventricle end-systolic volume, RVEF=right ventricle end-systolic volume, RVSV=right ventricle stroke volume, RVCO= right ventricular cardiac output, DCE= dynamic contrast enhanced imaging, FWHM=full-width half maximum

RESPIRE study: Sensitivity and repeatability of non-invasive endpoints

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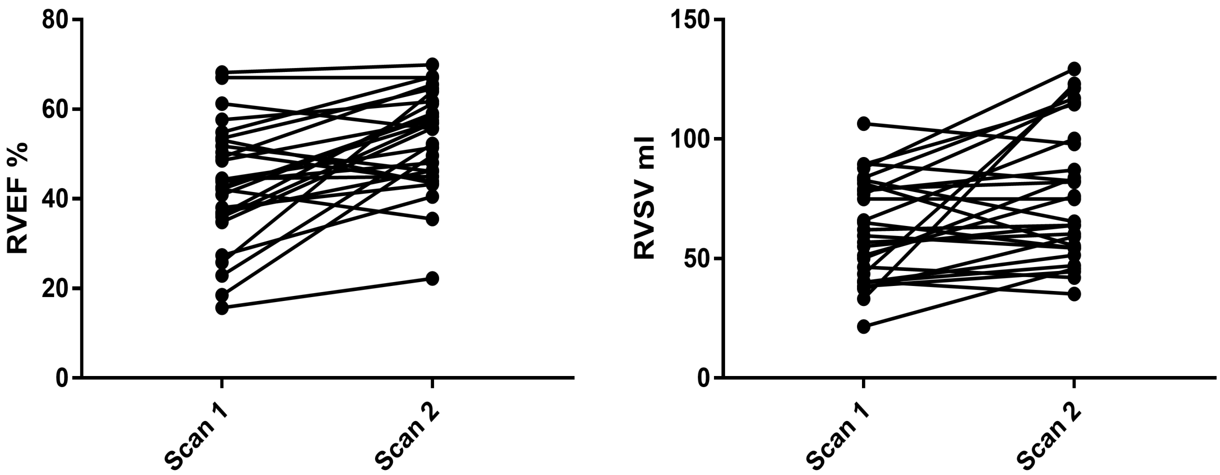
80
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40
20
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RVEF %
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Scan 2
150
100
50
0
RVSV ml
Scan 1
Scan 2

Supplementary file S1

Methods

Patients

Patients with PAH were prospectively recruited from a nationally designated pulmonary hypertension referral centre. We aimed to recruit 3 approximately equal size groups of patients: incident treatment-naïve patients commencing therapy, prevalent patients escalating therapy and patients who were deemed to be clinically stable and required no escalation of therapy. For study inclusion, patients were required to have idiopathic pulmonary arterial hypertension (IPAH), heritable PAH, PAH associated with connective tissue disease or portal hypertension, to have undergone RHC and have a mean pulmonary arterial pressure ≥ 25 mmHg and pulmonary arterial wedge pressure ≤ 15 mmHg and to have had other causes of pulmonary hypertension excluded. Exclusion criteria included pregnancy, allergy to contrast medium, contraindication to MRI or known active Hepatitis B or C or HIV infection. Ethical and institutional review board approval was obtained and all patients provided informed written consent.

Study investigations

Investigations performed at visit 1 included a blood draw (NT-ProBNP), an exercise test (6MWT) and MRI. Any subsequent treatment initiation or escalation occurred after visit 1 investigations were completed. Follow-up visits 2 and 3 occurred approximately 6 months after study visit 1. Visits 2 and 3 occurred within 24 hours of each other. Typically, visit 2 investigations occurred in the morning, and visit 3 investigations occurred in the afternoon or the following day. The study flow diagram, **Supplementary figure S2**, describes the study investigations. Patients took their usual medications approximately 1 hour prior to their MRI. Patients rested for 45 minutes after the 6-minute walking test to allow for recovery as per ATS recommendations. Patients were asked to refrain from exercise, caffeinated drinks and alcohol between investigations during their visits. Follow up scans at visits 2 and 3 were performed on the same MRI system as at visit 1.

MRI acquisition

The rationale for performing measurements on two different MRI scanners at different field strengths and from different vendors was to assess the variation introduced in the candidate MRI measurements across platforms. Short axis cine cardiac imaging stacks were performed using a balanced single shot free precession sequence with full ventricular coverage. End-systole was defined as the smallest chamber area on each slice and end-diastole was defined as the first cine phase of the R-wave triggered acquisition. The phase-contrast velocity imaging was performed 1cm above and orthogonal to the pulmonary valve. For dynamic contrast enhanced perfusion imaging a three-dimensional spoiled gradient echo time-resolved Magnetic Resonance Angiography sequence was used, positioned coronal across the chest with full lung coverage with an intravenous bolus injection of Gadovist (Bayer Healthcare, Germany) (0.05 mL/kg at 4ml/s followed by a saline flush of 20mL). Sequence parameters were: TR=2.1ms, T=0.7ms, FA=30°, BW=250kHz, with a reconstructed voxel size of 1.875x1.875x10mm and an effective frame rate of ~0.5s per whole lung volume.

MRI analysis

Epicardial and endocardial contours were drawn using semi-automatic tools with intensity thresholding of the structures within the endocardial contour, and biventricular volume, mass and function recorded. Regions of interest were drawn on the pulmonary artery and left atrium of the dynamic contrast enhanced perfusion images to calculate first pass pulmonary transit time (PTT) and full-width-half-maximum (FWHM) using in-house software. See **Supplementary figure S3**

Statistical analysis

Following histogram analysis, variables were categorised into normal and non-normal distributions: if normally distributed, mean and standard deviation were presented, whereas if non-normally distributed, median and interquartile range were presented.

Results

Patients

A total of 104 patients with PAH or suspected PAH were approached to take part in the study, 58 patients with suspected PAH were consented; of the 58 patients, 44 met the entry criteria after completion of their baseline study investigations and 42 completed the study. Patients had a mean age of 51 years and 83% were female. **Supplementary Table S5** presents demographic, pulmonary function test and right heart catheter data for all patients. Two patients experienced some claustrophobic symptoms at both visits but completed the study protocol. The mean time intervals between baseline and follow up for these 3 subgroups were: 6 months (standard deviation (SD) 3 months), 6 months (SD 1 month), and 8 months (SD 2 months) respectively. MRI and 6MWT were normally distributed on visual inspection of histograms. NT-ProBNP had a skewed distribution, however, following $\log_{10}$ transformation the distribution was normalised.

Supplementary figures

S2 Study flow chart illustrating the three study visits and mandatory investigations. All patients and healthy volunteers included in the study underwent the same protocol

S3 Illustration of the diastolic (A) and systolic (B) short axis cardiac cine MRI (epicardial and endocardial contours drawn and thresholding applied). Magnitude (C) and flow (D) images from phase contrast MRI sequence placed orthogonal to the main pulmonary artery. Dynamic contrast enhanced images illustrating contrast in the pulmonary arteries (E) and passing through to the left atrium, left ventricle and aorta (F).

S4 Line plots showing the changes in right ventricular ejection fraction (RVEF) and right ventricular stroke volume (RVSV) for individual patients in the combined initiating and escalating therapy group.

Supplementary tables

Supplementary table S5: Demographics, therapy, diagnostic and right heart catheter data in patients with PAH.

	All patients n=42	Treatment naïve n=16	Treatment change n=12	Stable n=14
Demographics				
Age (yrs)	51 (15)	54 (14)	52 (16)	46 (15)
Female [n (%)]	35 (83)	14 (88)	10 (83)	11 (79)
WHO functional class	I (0) II (2) III (35) IV (5)	I (0) II (0) III (13) IV (3)	I (0) II (0) III (11) IV (1)	I (0) II (2) III (11) IV (1)
Treatment (visit 1 therapy)				
Monotherapy	10 (24%)	0 (0%)	8 (67%)	2 (14%)
Combination therapy	10 (24%)	0 (0%)	4 (33%)	6 (43%)
Parenteral prostanoid	6 (14%)	0 (0%)	0 (0%)	6 (43%)
Calcium channel blocker	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Treatment (visit 2 therapy)				
Monotherapy	4 (10%)	1 (6%)	1 (8%)	2 (14%)
Combination oral therapy	23 (54%)	9 (56%)	8 (67%)	6 (43%)
Parenteral prostanoid	13 (31%)	4 (25%)	3 (25%)	6 (43%)
Calcium channel blocker	2 (5%)	2 (13%)	0 (0%)	0 (0%)
Subgroups				
IPAH and HPAH	25 (60.9%)	8 (50%)	8 (66.6%)	10 (71.1%)
PAH CTD	10 (24.4%)	6 (37.5%)	2 (16.6%)	2 (14.3%)
PAH congenital	1 (2.4%)	1 (6.25%)	0 (0%)	0 (0%)
PAH portal	4 (10%)	1 (6.25%)	1 (8.3%)	2 (14.3%)
PAH drug and toxins	1 (2.4%)	0 (0%)	1 (8.3%)	0 (0%)
Walk test				
6MWT Distance (m)	375 (167)	303 (144)	332 (179)	488 (120)
Blood tests				
Log_{10}NT-ProBNP*	561 (258 to 906)	619 (213 to 1095)	665 (301 to 1356)	386 (176 to 606)
Right heart catheter				
mRAP (mmHg)	11 (7)	13 (7)	14 (7)	6 (2)
mPAP (mmHg)	52 (10)	53 (7)	58 (10)	46 (14)
PCWP (mmHg)	10 (3)	10 (2)	12 (2)	10 (3)
CI (L/min/m2)	2.4 (0.9)	2.0 (0.8)	2.0 (0.7)	3.0 (0.8)
PVR (Wood units)	11 (6)	14 (6)	13 (5)	7 (5)
Mixed venous oxygen saturations (%)	64 (10)	60 (11)	64 (6)	69 (7)

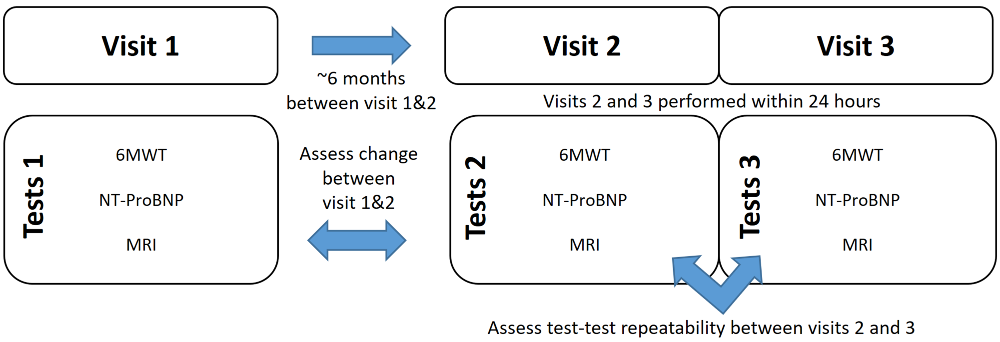
Values are mean (SD) or n (%) unless otherwise stated
* Median (IQR) presented

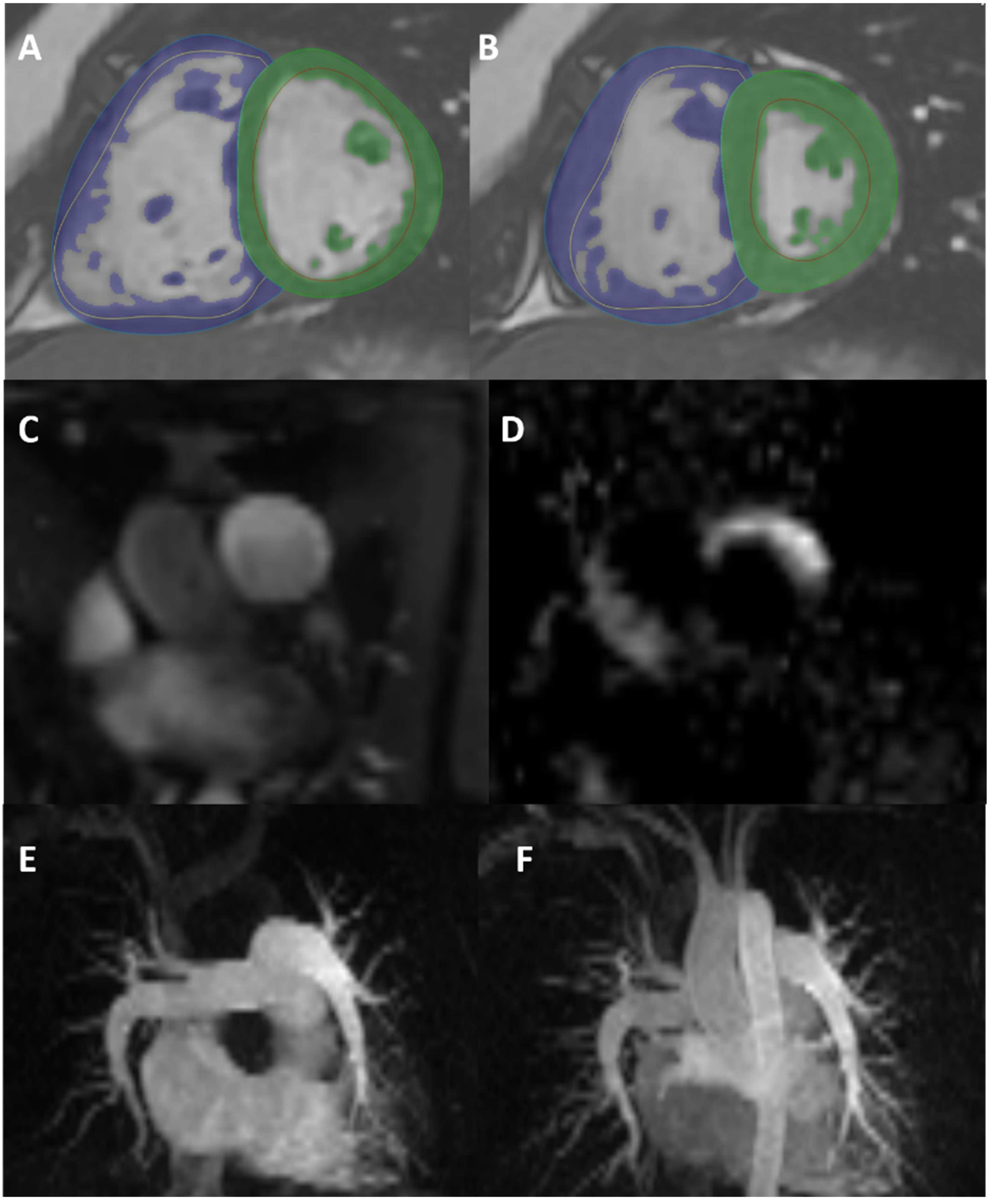
Supplementary table S6 Mean and standard deviation of walk test, NT-ProBNP and MRI metrics in stable patients with PAH who do have no change in PAH therapy between visit 1 or visit 2.

	N	Visit 1		Visit 2		Change (Visit 1 – Visit 2)			95% Confidence Interval		Cohen's d
		Mean	SD	Mean	SD	Mean difference	SD	SEM	Lower	Upper	
Walk test											
6MWT Distance (m)	12	479.83	121.47	473.50	117.98	6.33	80.62	23.27	-44.89	57.56	0.05
Blood test											
Log NT-ProBNP	14	2.53	0.44	2.67	0.32	-0.14	0.37	0.10	-0.35	0.08	0.36
SA with threshold											
RVEDM (g)	14	85.34	27.13	82.55	26.41	2.79	10.47	2.80	-3.25	8.83	0.10
RVESM (g)	14	72.00	24.77	75.33	30.28	-3.33	13.21	3.53	-10.96	4.30	0.12
RVEDV (ml)	14	97.89	31.92	105.75	41.23	-7.86	23.09	6.17	-21.19	5.47	0.21
RVESV (ml)	14	49.18	20.47	52.07	19.88	-2.89	9.26	2.47	-8.24	2.45	0.14
RVEF (%)	14	50.45	8.51	49.58	11.01	0.87	13.54	3.62	-6.95	8.69	0.09
RVSV (ml)	14	48.70	15.29	53.67	26.94	-4.97	25.42	6.79	-19.65	9.71	0.23
RVCO (L/min)	14	3.27	1.12	3.60	2.06	-0.33	1.74	0.47	-1.34	0.68	0.20
Systolic septal angle (°)	13	149.69	16.24	153.00	12.01	-3.31	14.44	4.00	-12.03	5.42	0.23
Diastolic septal angle(°)	13	142.31	10.56	143.15	9.11	-0.85	5.76	1.60	-4.32	2.63	0.09
Q flow											
Net flow volume (ml)	13	79.94	55.65	72.38	32.66	7.56	45.72	12.68	-20.07	35.18	0.17
Forward flow volume (ml)	13	82.17	53.54	74.39	30.50	7.78	43.20	11.98	-18.33	33.89	0.18
Backward flow volume (ml)	13	2.24	5.05	2.02	3.83	0.22	4.85	1.35	-2.71	3.15	0.05
Regurgitant fraction (%)	13	5.67	12.77	4.87	10.24	0.81	12.74	3.53	-6.89	8.51	0.07
Average flow velocity (cm/s)	13	7.97	4.10	8.74	4.57	-0.78	3.00	0.83	-2.59	1.04	0.18
Peak flow velocity (cm/2)	13	66.06	32.37	69.33	29.30	-3.27	39.47	10.95	-27.12	20.58	0.11
Diastolic vessel area (mm2)	13	988.46	314.83	916.21	290.08	72.26	241.04	66.85	-73.40	217.91	0.24
Systolic vessel area (mm2)	13	1110.66	305.79	1042.87	287.10	67.78	245.12	67.98	-80.34	215.91	0.23
Pulmonary arterial pulsatility (%)	13	13.98	6.78	15.25	7.02	-1.27	5.42	1.50	-4.55	2.01	0.18
DCE imaging											
Pulmonary Transit Time (s)	11	5.67	1.13	5.91	1.67	-0.25	1.34	0.40	-1.15	0.65	0.17
FWHM (s)	11	5.21	1.68	5.02	1.50	0.20	1.66	0.50	-0.92	1.31	0.12

6MWT= six minute walk test, Log10NT-ProBNP=log to base 10 N Terminal brain naturitic peptide, SA= short axis, RVEDM=right ventricle end-diastolic mass, RVESM=right ventricle end-systolic mass, RVEDV=right ventricle end-systolic volume, RVEF=right ventricle end-systolic volume, RVSV=right ventricle stroke volume, RVCO= right ventricular cardiac output, DCE= dynamic contrast enhanced imaging, FWHM=full-width half maximum

RESPIRE study: Sensitivity and repeatability of non-invasive endpoints

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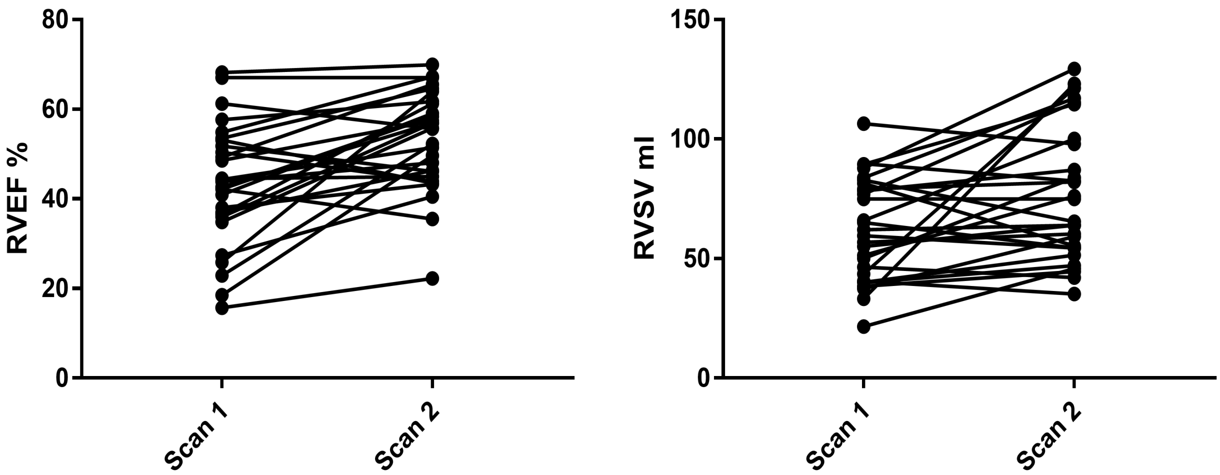
80
60
40
20
0
RVEF %
Scan 1
Scan 2
150
100
50
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RVSV ml
Scan 1
Scan 2