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ORIGINAL RESEARCH ARTICLE

Sequential nephron blockade with combined diuretics improves diastolic function in patients with resistant hypertension

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Abstract

Aims Hypertension is a major contributor to cardiac diastolic dysfunction. Different therapeutics strategies have been proposed to control blood pressure (BP), but their independent impact on cardiac function remains undetermined. In patients with resistant hypertension, we compared the changes in cardiac parameters between two strategies based on sequential nephron blockade (NBD) with a combination of diuretics or sequential renin-angiotensin system blockade (RASB).

Methods and results After a 4-week period where all patients received Irbesartan 300 mg/day + hydrochlorothiazide 12.5 mg/day + amlodipine 5 mg/day, 140 resistant hypertension patients (54.8 ± 11.1 years, 76% men, mean duration with hypertension: 13.1 ± 10.5 years, no previous history of heart failure or current symptoms of congestive heart failure) were randomized 1:1 to the NBD regimen or to the RASB regimen at week 0 (W0, baseline). Treatment intensity was increased at week 4, 8, or 10 if home BP was ≥135/85 mmHg, by sequentially adding 25 mg spironolactone, 20–40 mg furosemide, and 5 mg amiloride (NBD group) or 5–10 mg ramipril and 5–10 mg bisoprolol (RASB group). No other antihypertensive drug was allowed during the study. BP, BNP levels, and echocardiographic parameters were assessed at weeks 0 and 12.

The baseline characteristics, laboratory parameters, and plasma hormones (BNP, renin, and aldosterone) and cardiac echocardiographic parameters did not significantly differ between the NBD and the RASB groups. Over 12 weeks, BNP levels significantly decreased in NBD but increased in RASB (mean [CI 95%] change in log-transformed BNP levels: −43% [−67%; −23%] vs. +55% [46%; 62%] in NBD vs. RASB, respectively, $P < 0.0001$). Similarly, the proportion of patients presenting ≥2 echocardiographic criteria of diastolic dysfunction decreased between baseline and W12 from 31% to 3% in NBD but increased from 19% to 32% in RASB ($P = 0.0048$). As compared with RASB, NBD induced greater decrease in ambulatory systolic BP ($P < 0.0001$), pulse pressure ($P < 0.0001$), and systemic vascular resistance ($P < 0.005$). In multivariable linear regression analyses, NBD treatment was significantly associated with decreased BNP levels (adjusted *ß*: −46.41 ± 6.99, $P < 0.0001$) independent of age, gender, renal function, and changes in BPs or heart rate.

Conclusions In patients with resistant hypertension, nephron blockade with a combination of diuretics significantly improves cardiac markers of diastolic dysfunction independently of BP lowering.

Keywords Heart failure; Resistant hypertension; Pharmacology; Clinical trials

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Introduction

Heart failure with preserved ejection fraction (HFpEF) has been reported to account for almost half of all heart failure (HF) patients. HFpEF typically occurs in association with advanced age, cardiovascular, metabolic, and proinflammatory comorbidities.[1] One of the most common comorbidities associated with HFpEF is arterial hypertension, which is

thought to favour the development of left ventricular (LV) stiffening, hypertrophy, and consequent diastolic dysfunction.[2] Patients with resistant hypertension (RHTN), defined as a failure to reach seated office systolic blood pressure $<$ 140 mmHg or diastolic blood pressure $<$ 90 mmHg while treated with an appropriate three drug regimen that includes a diuretic have a higher risk to develop HF, notably HFpEF.[3,4]

Consequently, current European and U.S. guidelines recommend treating hypertension in HFpEF patients with diuretics, angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin-II-receptor blockers (ARBs), mineralocorticoid receptor antagonists (MRAs), or beta-blockers.[3] These anti-hypertensive drugs have different pharmacological targets with potential impact on myocardial remodelling or on the further development of cardiac dysfunction.[3] For instance, one of the major contributors to RHTN is fluid retention, which also contributes to increase LV filling pressures.[5,6] Aldosterone blockade with mineralocorticoid receptor antagonist has been shown to reduce myocardial fibrosis, an important component observed in HFpEF.[7,8] Reciprocally, the use of beta-blockers in HFpEF patients is debated as potentially associated with increased risk of cardiovascular outcomes.[9] The optimal antihypertensive strategy in patients with RHTN to both treat hypertension and improve cardiac diastolic dysfunction is thus currently undetermined.

The 'Management of Resistant Hypertension: Comparison of Two Treatment Strategies: Increase Sodium Depletion or Combined Blockage of Renin-angiotensin System (RAS)' ('PHARES trial', NCT00224549)[10] compared the efficacy of two different anti-hypertensive pharmacological strategies in RHTN patients. The primary results showed that sequential nephron blockade (NBD) with different diuretics (i.e. furosemide, spironolactone, and amiloride) acting on three segments of renal tubule of the nephron induced a large and well-tolerated reduction in BP compared with a strategy with combined renin-angiotensin system blockade (RASB) with irbesartan, ramipril, and bisoprolol.

In this pre-specified substudy of the PHARES trial, we aimed to evaluate and compare the changes in cardiac biomarkers (BNP) and echocardiographic parameters of diastolic dysfunction according to the two anti-hypertensive strategies in RHTN patients.

Methods

Study design

The design of this 12-week, single-centre, prospective, randomized, open, blinded endpoint trial with optional drug titration has been described elsewhere[10] and will be summarized briefly here. The study was approved by the Ethics Committee of Cochin hospital in Paris and is registered with Clinicaltrials.gov NCT00224549. All patients provided written informed consent, and all procedures followed were in accordance with institutional guidelines and the Declaration of Helsinki.

Briefly, eligible men or women were aged 18 to 75 years, with essential hypertension resistant to three or more antihypertensive drugs including a diuretic (supine office blood pressure BP at least 140 and or 90 mmHg). The main exclusion criteria were secondary hypertension, history of severe cardiovascular disease (cardiac surgery or percutaneous coronary angioplasty), or stroke in the past 3 months, atrial fibrillation, uncontrolled diabetes (HbA1c $>$ 8%), and estimated glomerular filtration rates less than 40 mL/min (modification of diet in renal disease formula). The patients entered a 4-week standardized triple-therapy regimen comprising hydrochlorothiazide (12.5 mg/day), irbesartan (300 mg/day), and amlodipine (5 mg/day) (*Figure 1A*). After 4 weeks of the standardized triple-therapy regimen, patients with a mean daytime ambulatory systolic blood pressure $\geq$ 135 mmHg and/or mean daytime ambulatory diastolic blood pressure $\geq$ 85 mmHg were randomized 1:1 to the NBD regimen or to the RASB regimen at week 0 (W0, baseline). Treatment intensity was increased at week 4, 8, or 10 if home BP was $\geq$135/85 mmHg, by sequentially adding 25 mg spironolactone, 20–40 mg furosemide, and 5 mg amiloride (NBD group) or 5–10 mg ramipril and 5–10 mg bisoprolol (RASB group). No other antihypertensive drug was allowed during the study.

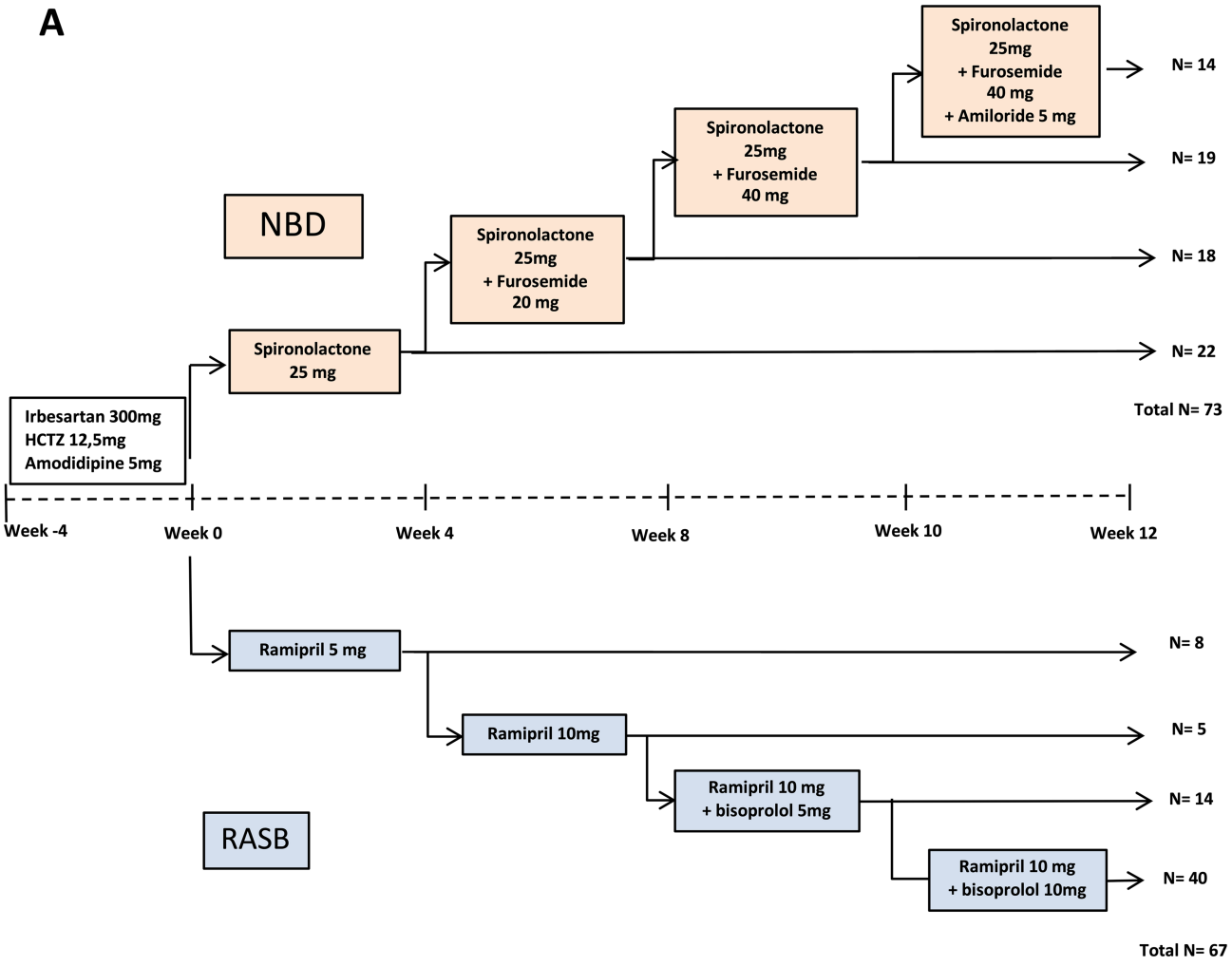
At W4, W8, W10, and W12, patients reported to the centre at approximately 0830 h, without having taken their morning dose, to undergo safety, office BP, and laboratory assessments.

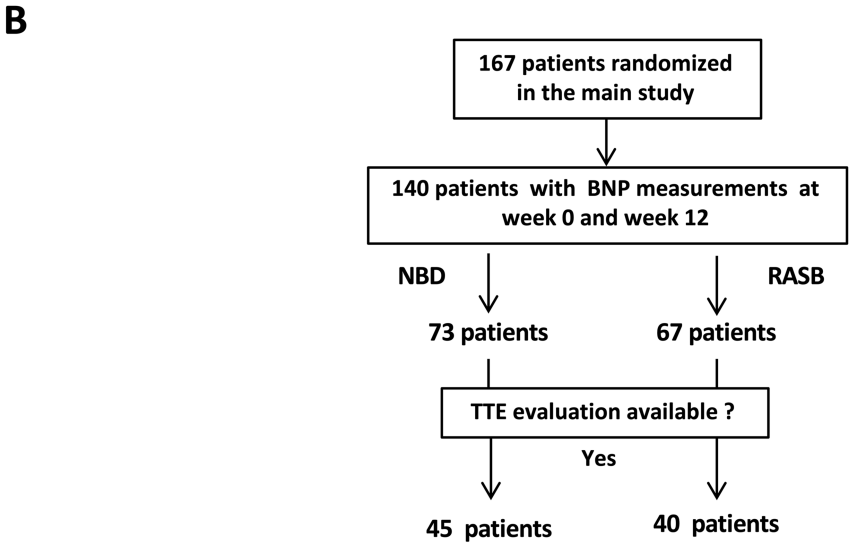
Evaluation of cardiac biomarkers and diastolic function

BNP levels were measured at baseline and at the 12-week follow-up visit. The cut-offs BNP levels were defined in line with current guidelines in non-acute settings: BNP levels above 100 pg/mL were considered as likely indicating the presence of HF while BNP levels below 35 pg/mL were considered as invalidating the presence of HF.[1] The grey zone was comprised between these two limits.

Trans-thoracic echocardiography was also performed at baseline (W0) and at the last follow-up visit (W12) with a Vivid 7 ultrasound system (General Electric Vivid 7, GE Health care) and by the same experienced operator blind to the randomization group, as described previously.[11] Cardiac dimensions including wall thickness, ventricular diameters, and left atrial area were measured in accordance with the American society of the echocardiography guideline.[12] Pulse wave

FIGURE 1 Design of the study.





Doppler recordings of the LV inflow were obtained from the apical four chambers view with the simple volume place between the types of the mitral leaflets. Tissue Doppler was used to measure septal and lateral velocity of the mitral annulus. Maximal velocity of tricuspid insufficiency and left atrial area in four chamber apical view were also assessed. According to the European Society of Cardiology and American Society of Echocardiography guidelines, left atrial area, septal, and lateral E wave velocity, E/E′ ratio and tricuspid insufficiency velocity were measured to assess cardiac diastolic function.[12,13] Abnormal measurements were considered for left atrial area $>20cm^2$, septal or lateral E wave measured by tissue Doppler <7 or <10 cm/s, respectively, E/é ratio $>$ 14 or tricuspid insufficiency velocity $>$ 2.8 m/s. Diastolic functions were considered as normal if zero or one criterion was met and undetermined if two criteria were met. Diastolic dysfunction was defined if ≥ 3 of these criteria were met.

Haemodynamic and vascular measurements

Supine office BP was measured with a validated electronic device (Omron M4; Omron Co., Kyoto, Japan), as previously described.[10] Twenty four-hour ambulatory, daytime (from 07:00 to 22:00) and nighttime (from 22:00 to 07:00) BP and heart rate (HR) monitoring was performed with Spacelabs 90207 monitor (Spacelabs Healthcare, Issaquah, Washington).[10]

Systemic vascular resistance (SVR) was calculated using the formula: SVR = (daytime mean arterial BP blood pressure − central venous pressure) × 79.92/cardiac output.

Mean arterial blood pressure was calculated with the formula: (daytime mean arterial systolic blood pressure + 2 × daytime mean arterial diastolic blood pressure). Cardiac output was estimated with the formula: $[\pi/2 \times (\text{LV outflow tract diameter})^2 \times \text{LV outflow tract sub-valvular velocity time integral})] \times \text{heart rate}$.

In line with the lack of clinical congestive signs in these patients, central venous pressure was estimated to 3 mmHg for all patients. Because none of the patients had clinical signs of congestive HF, central venous pressure was estimated to be 3 mmHg for all patients.[14]

Central BP and pulse wave velocity (PWV) were measured at baseline at W12 by aplanation tonometry using the Sphygmocor device (AtCor, Sydney, Australia) as described previously.[15,16] This system uses a single element tonometer applied on the radial artery. Then a validated transfer function is used to evaluate aortic BP. PWV was measured between the carotid and the femoral waveforms using the R-wave of the ECG for the synchronization and was calibrated using the 0.8× factor.[15,16]

Laboratory parameters

Blood was sampled at baseline and at the last follow-up visit in fasting conditions at ≈09:00 after 30 min rest in supine position to measure plasma renin, aldosterone, BNP, creatinine, sodium, potassium, and protein concentrations. Plasma renin concentration was determined using the RENIN III GENERATION radioimmunometric assay kit from Cisbio Bioassays (Bedford, MA, USA). Plasma concentration of aldosterone was determined using the solid-phase ^{125}I radioimmunoassay kit 'Coat-A-Count® Aldosterone' from Siemens (Los Angeles, CA, USA). Plasma BNP concentration was measured using a Chemiluminescence Dx1800 (Beckman Coulter) technique. All BNP values below the detection limit of 1 pg/mL were set to 1 pg/mL.

Statistical analysis

Continuous variables are expressed as means ± standard deviations for variables normally distributed and median [Q1 Q3 interquartile range] for others. Categorical variables are expressed as numbers and percentages. A Student's *t*-test was used for continuous variable and a $\chi 2$ square test or Fisher's exact test for categorical variable was performed for comparison between groups and between baseline and week 12. Statistical results are presented as mean differences, with two-tailed 95% confidence intervals for variables normally distributed or variation as percent with two-tailed 95% confidence intervals for others.

The main endpoint was the absolute change in BNP levels from baseline to week 12 in both groups. For BNP values under the limit of detection (1 pg/mL), BNP was set at this value. Factors influencing the variation over 12 weeks of log-transformed BNP were assessed using univariable and multivariable linear regression analyses. In addition to age and gender, the factors related at the $P < 0.1$ level were further selected for multivariable analyses and corresponding beta coefficient, standard deviations, and *P* values were reported.

R (version 3.5.0) was used for statistical analysis. To adjust for multiple testing, a *P* value < 0.01 was considered statistically significant.

Results

Patients characteristics at randomization

Among the 167 patients randomized for the main study, BNP levels were available at both randomization and the 12-week follow-up visit for 140 patients: 73 patients randomized to the NBD group and 67 to RASB group (total 140 patients,

Figure 1). The baseline characteristics, laboratory parameters, and plasma hormones (BNP, renin, and aldosterone) did not significantly differ between the NBD and the RASB groups (*Tables 1* and *2*). None of these patients had previous history of HF or current symptoms of congestive HF.

The proportion of patients with baseline plasma BNP concentrations $<$ 35 pg/mL, between 35 and 100 pg/mL, and $>$100 pg/mL did not significantly differ between the two groups (*Figure 2A* and *2B*). Only three (4.1%) patients of the NBD group and one (1.5%) of the RASB group had baseline plasma BNP concentrations $>$ 100 pg/mL.

Among the 140 patients with BNP measurements, 45 patients in NBD group and 40 in RASB group had TTE recordings available for analysis (*Figure 1B*). At baseline, there was no significant difference in echocardiographic parameters between the two groups (*Table 3*). The mean LVMI in men were 103.3 ± 21.2 g/m^2 in NBD group and 100.8 ± 26.6 g/m^2 in RASB group (P = 0.573), and in women, 95.3 ± 21.5 g/m^2 in NBD group and 92.5 ± 19.7 g/m^2 in RASB group, (P = 0.765). A total of 21 patients (11 women and 10 men) had LV hypertrophy. The echocardiographic parameters required to score diastolic dysfunction were available in 32 patients of the NBD and 31 patients of the RASB arm. Nine patients in NBD group and five patients in RASB group had undetermined level of diastolic function, and one patient in each group had echocardiographic diastolic dysfunction (P = 0.676, *Table 3*, *Figure 2C* and *2D*).

Table 1 Baseline characteristics of patients with resistant hypertension randomized to nephron blockade or renin-angiotensin system blockade group and complete BNP evaluation

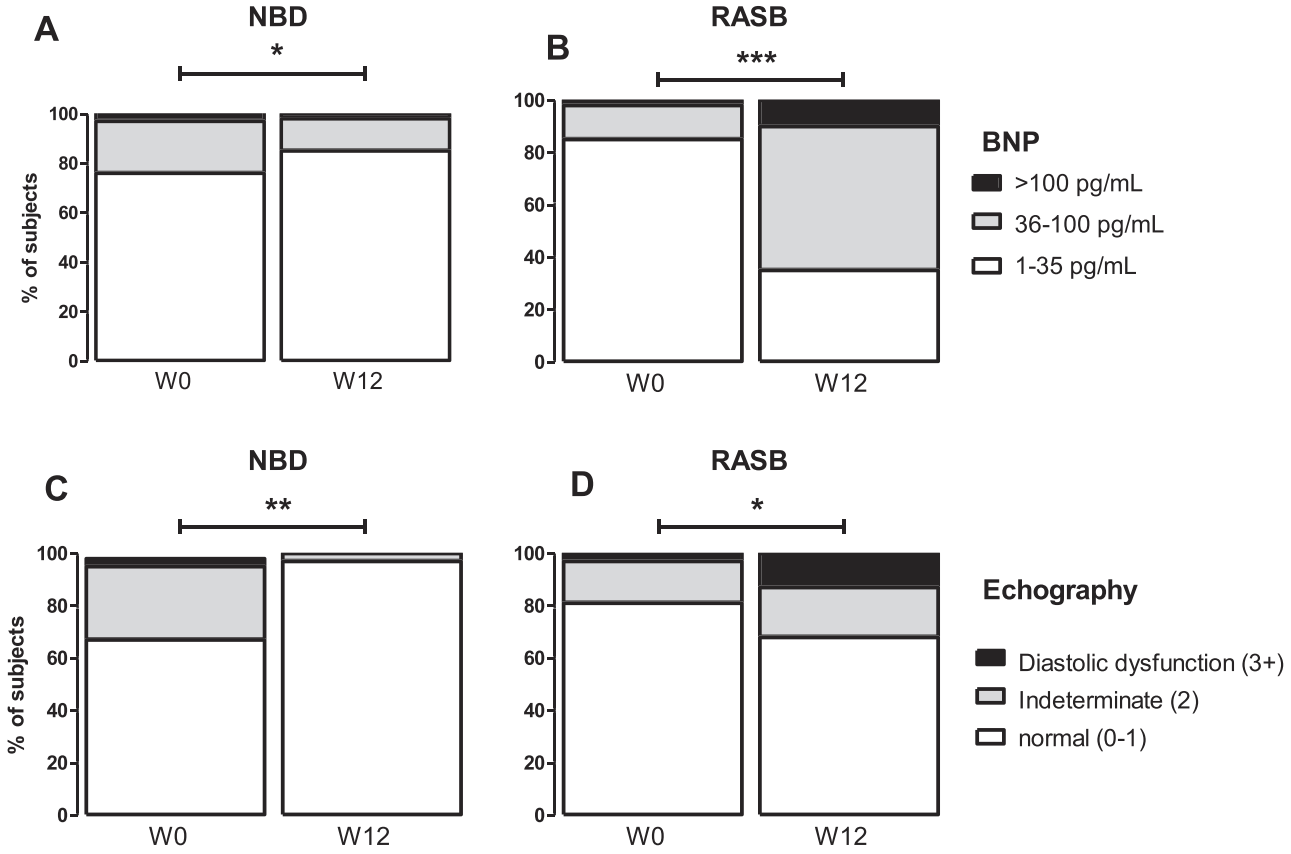
n = 140	NBD group n = 73	RASB group n = 67	P
Age (years)	55.8 ± 10	53.7 ± 10.3	0.233
Men, n (%)	54 (74)	53 (79)	0.475
BMI (kg/m^2)	30.0 ± 4.9	28.3 ± 3.8	0.031
Obesity (BMI $>$ 30 kg/m^2), n (%)	32 (44)	24(36)	0.334
Diabetes mellitus, n (%)	12 (15)	15 (22)	0.609
Dyslipidemia, n (%)	46 (63)	40 (60)	0.326
Duration of hypertension (years)	14.3 ± 10.3	12.7 ± 10.6	0.352
Daytime ambulatory SBP (mmHg)	148.7 ± 12.4	149.9 ± 12.1	0.569
Daytime ambulatory DBP (mmHg)	91.4 ± 10.1	93.3 ± 9.3	0.245
Daytime ambulatory PP (mmHg)	57.4 ± 10.6	56.6 ± 11.2	0.694
Daytime ambulatory HR (bpm)	79.6 ± 9.9	81.7 ± 10.2	0.375
Night-time ambulatory SBP (mmHg)	137.2 ± 13.1	135.4 ± 13.5	0.419
Night-time ambulatory DBP (mmHg)	81.0 ± 9.9	80.9 ± 9.5	0.941
Night-time ambulatory HR (bpm)	70.3 ± 8.8	69 ± 9.9	0.446
Night-time ambulatory PP (mmHg)	56.2 ± 10.2	54.5 ± 10.5	0.332
Pulse wave velocity (m/s)	10.9 ± 2.1	10.9 ± 1.9	0.949

BMI, body mass index; DBP, diastolic blood pressure; PP, pulse pressure; SBP, systolic blood pressure.
Results are mean ± SD or mediane [IQR].

Table 2 Comparison between the two arms of treatment of haemodynamic and biological parameters at weeks 0 and 12

	Week 0			Week 12		
	NBD (n = 73)	RASB (n = 67)	P Value	NBD (n = 73)	RASB (n = 67)	P Value
Plasma BNP (pg/mL)	21 [11–33]	17 [10–27]	0.216	12 [8–22]	40 [17–64]	$<$0.0001
Daytime ambulatory SBP (mmHg)	148.7 ± 12.4	149.9 ± 12.1	0.569	128.0 ± 11.7	142.1 ± 17.7	$<$0.0001
Daytime ambulatory DBP (mmHg)	91.4 ± 10.1	93.3 ± 9.3	0.245	79.5 ± 9.9	86.1 ± 9.9	0.0011
Daytime ambulatory PP (mmHg)	57.4 ± 10.6	54.0 ± 14.7	0.119	48.1 ± 10.1	56.0 ± 11.2	$<$0.0001
Aortic SBP (mmHg)	129.6 ± 19.8	127.4 ± 16.8	0.525	102.5 ± 17.3	118.3 ± 18.4	$<$0.0001
Aortic PP (mmHg)	48.0 ± 14.6	47.0 ± 12.7	0.683	35.5 ± 12.3	46.0 ± 13.3	$<$0.0001
Pulse wave velocity (m/s)	10.9 ± 2.1	10.9 ± 1.9	0.949	9.9 ± 1.9	10.3 ± 2.0	0.197
Systemic vascular resistance (Wood)	3064.9 ± 856.3	3131.5 ± 786.8	0.687	1396.5 ± 382.6	1642.3 ± 383.4	0.0024
Daytime ambulatory HR (bpm)	79.6 ± 9.9	81.7 ± 10.2	0.375	80.9 ± 9.8	68.4 ± 11.8	$<$0.0001
Plasma potassium (mmol/L)	3.8 ± 0.4	3.9 ± 0.4	0.199	4.3 ± 0.5	4.0 ± 0.4	$<$0.0001
Plasma sodium (mmol/L)	140.6 ± 2.2	140.8 ± 2.4	0.682	138 ± 3	140 ± 2	$<$0.0001
eGFR (ml/min per 1.73m^2)	83.5 ± 18.6	89.7 ± 18.9	0.055	73 ± 20	86 ± 18	$<$0.0001
Plasma proteins (g/L)	69.5 ± 4.9	68.2 ± 4.2	0.122	70 ± 5	68 ± 4	0.0018
Plasma renin (mUI/L)	19 [10–39]	20.5 [9–37.8]	0.922	122 [52–327]	8 [5–29]	$<$0.0001
Plasma aldosterone (pmol/L)	104 [69–152]	95[63.3–132]	0.362	270 [177–344]	91[58–137]	$<$0.0001

FIGURE 2 Evolution from randomization (W0) to week 12 (W12) of BNP and echocardiographic parameters of diastolic dysfunction in nephron blockade and renin-angiotensin system blockade arms of treatment.



After 12 weeks, nephron blockade and renin-angiotensin system blockade had different effects on BNP levels

At 12 weeks, plasma BNP concentrations decreased significantly from baseline in the NBD group but increased in the RASB group (mean change in log-transformed BNP from W0 to W12: −43%, 95% CI [−67%; −23%] vs. +55%, 95% CI [+46%; +62%], respectively, $P < 0.0001$, *Figure 3* and *Table 2*). Consequently, the number of patients with plasma BNP concentrations between 35 and 100 pg/mL and >100 pg/ml decreased from baseline to W0 to nine (12%) and one (1.4%) patients in the NBD group, respectively, whereas it increased to 37 (55%) and 7 (10.4%) patients in the RASB group, respectively ($P < 0.001$; *Figure 2A* and *2B*).

In the univariate analysis, NBD treatment was significantly associated with decrease in BNP levels over the 12 weeks of the study (unadjusted *ß*: −42.51 ± 6.50, $P < 0.0001$, *Table 4*). The influence of NBD treatment was also significant after adjustment on classical factors influencing BNP levels including age, sex, renal function, and BMI (adjusted *ß*: −46.41 ± 6.99, $P < 0.0001$).

Nephron blockade has an independent effect on changes in BNP levels

We further looked at haemodynamics differences between the two groups that could explain the observed difference in BNP changes (*Table 2*). At the end of the study (week 12), the mean decrease from baseline in daytime systolic BP, diastolic BP, aortic systolic BP, and pulse pressure (PP) was significantly higher in the NBD group as compared with the RASB group (*Table 2*), in line with previous results in the main study. PWV tended to decrease in both groups, a trend that however did not reach significance. SVR were significantly lower after 12 weeks in the NBD arm compared with the RASB arm (*Table 2*). HR was significantly lower in the RASB group than the NBD group due to bisoprolol's effect.

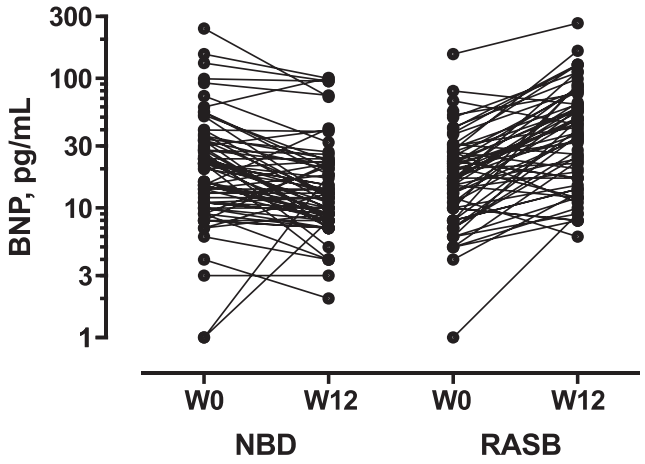
We next performed a multivariate analysis to identify significant factors associated with changes in BNP levels over the 12-week follow-up (*Table 4*). We found that higher age and lower changes in PP and in PWV over 12 weeks were significantly associated with smaller changes in BNP levels (*Table 4*). Reciprocally, NBD treatment was associated with larger

Table 3 Echocardiographic parameters at randomization (week 0) and after 12 weeks of nephron blockade or renin-angiotensin system blockade treatment

	Week 0			Week 12		
	NBD $N = 45$	RAS $N = 40$	*P* value	NBD $n = 45$	RAS $n = 40$	*P* value
LVED diameter (mm)	48.3 ± 5.2	50.7 ± 4.2	0.0244	48.4 ± 4.6	51.6 ± 5.8	0.0059
LVMI (g/m^2)	97.3 ± 17.5	98.2 ± 27.6	0.854	89.1 ± 18.9	100.0 ± 24.0	0.0246
LVEF (%)	68.0 ± 8.2	66.1 ± 8.5	0.299	66.9 ± 8.1	67.0 ± 7.5	0.968
Cardiac output (l/min)	6.1 ± 1.7	5.8 ± 1.4	0.529	5.8 ± 1.5	5.3 ± 1.2	0.083
LVED volume (mL/m^2)	65.9 ± 18.7	63.6 ± 11.9	0.532	66.6 ± 17.2	66.7 ± 16.6	0.977
LVES volume (mL/m^2)	20.9 ± 8.3	21.9 ± 7.8	0.589	22.4 ± 9.6	22.2 ± 8.6	0.922
TI velocity (cm/s)	2.46 ± 0.31	2.33 ± 0.23	0.0476	2.35 ± 0.21	2.47 ± 0.27	0.0327
Left atrial area (cm^2)	17.7 ± 4.2	18.5 ± 4.0	0.401	16.5 ± 3.6	19.2 ± 3.9	0.0021
Lat e-wave velocity (cm/s)	9.0 ± 2.3	8.7 ± 3.0	0.608	9.4 ± 2.3	9.1 ± 3.1	0.641
Sep e-wave velocity (cm/s)	7.0 ± 2.0	6.8 ± 1.9	0.564	7.5 ± 2.3	7.3 ± 2.5	0.789
E-wave velocity (cm/s)	69.9 ± 13.4	66.6 ± 15.8	0.302	67.5 ± 13.1	75.8 ± 20.5	0.0331
E/é ratio	9.6 ± 2.5	9.6 ± 3.0	0.917	8.7 ± 2.2	10.4 ± 4.3	0.0236
	Assesment of diastolic function					
	NBD $n = 32$	RAS $n = 31$	*P* value	NBD $n = 32$	RAS $n = 31$	*P* value
Normal, *n* (%)	22	25	0.676	31	21	0.0048
Undetermined, *n* (%)	9	5		1	6	
Diastolic dysfunction, *n* (%)	1	1		0	4	

LVEDd, left ventricular end diastolic diameter; LVMI, left ventricular mass index; TI velocity, tricuspid insufficiency velocity.
Full data of echographic parameters for assessment of diastolic function were available in 43% and 46% of subjects in NBD arm and RASB arm, respectively.

FIGURE 3 Evolution of BNP levels at baseline and after 12 weeks according to the nephron blockade or renin-angiotensin system blockade blockade.



reductions in BNP levels. Lastly, patients experiencing the larger reduction in HR had the smallest reduction in BNP levels.

At week 12, patients in the NBD group had lower plasma sodium, higher potassium, and higher plasma creatinine resulting in lower estimated glomerular filtration rate than RASB group (*Table 2*). Similarly, renin and aldosterone plasma concentrations increased more in NBD than in RASB (*Table 2*). However, none of these factors has significant influence on changes in BNP levels in univariate or multivariable analyses.

Nephron blockade improved diastolic function assessed by echocardiography

After 12 weeks, we observed that LV diastolic diameter, LV mass index, and left atrial area decreased in NBD group, while these parameters slightly increased in the RASB group (*Table 3*). In both NBD and RASB, cardiac index did not change significantly between W0 and W12.

In line with our results on changes in BNP levels, we observed an improvement of diastolic parameters in NBD

Table 4 Factors influencing difference in BNP levels between week 12 and week 0 in univariable and multivariable linear regression

	Univariable		Multivariable	
	ß	*P* Value	*ß*	*P* value
Age (years)	0.17 ± 0.36	0.636	0.91 ± 0.33	0.0065
Women	−1.02 ± 8.76	0.907	4.59 ± 8.12	0.573
NBD versus RASB treatment	−42.51 ± 6.50	<0.0001	−20.91 ± 9.44	0.0291
Difference in daytime SBP (mmHg)	1.12 ± 0.24	<0.0001	−0.78 ± 0.55	0.161
Difference in daytime PP (mmHg)	2.67 ± 0.44	<0.0001	3.34 ± 1.06	0.0018
Difference in aortic SBP (mmHg)	0.73 ± 0.22	0.0013	0.19 ± 0.38	0.613
Difference in aortic PP (mmHg)	0.86 ß 0.34	0.0122	−0.78 ± 0.55	0.160
Difference in pulse Wave velocity (m/s)	4.55 ± 2.55	0.077	5.19 ± 2.0	0.0097
Difference in HR (bpm)	−1.68 ± 0.28	<0.0001	−0.97 ± 0.41	0.0186
Difference in systemic vascular resistance (Wood)	−0.01 ± 0.01	0.924		
eGFR (mL/min per 1.73m^2)	0.28 ± 0.18	0.134		
BMI (kg/m^2)	0.63 ± 0.84	0.455		

group: at week 12, 97% of patients had a normal diastolic function compared with 69% at baseline (P = 0.001). Only one patient in NBD group remained with undetermined diastolic function, none with diastolic dysfunction (*Table 3*, *Figure 2C*). Reciprocally, echography indicated worsening of diastolic function at week 12 in patients of the RASB group (*Figure 2D*). At week 12, only 68% of patients in RASB group presented normal diastolic function (*Table 3*, *Figure 2*).

Discussion

We report that in patients without symptomatic HF but with RHTN as a major risk factor for HF, a 12-week treatment based on sequential NBD with combined diuretics significantly and rapidly improves biological and echocardiographic markers of diastolic function while a strategy of triple RASB (with ARB, ACEI, and beta-blocker) did not improve these markers. This study was a pre-specified substudy of the PHARES trial, a prospective, randomized, open-blinded endpoint study, where 167 patients with mean baseline daytime ambulatory blood pressure of 135 mmHg or more and/or 85 mmHg or more despite 4-week treatment with irbesartan 300 mg/day, were randomized to NBD vs. RASB.[10] The PHARES study showed a significantly higher reduction in blood pressure and in LV mass in patients receiving NBD.[11] In this sub-study focusing on the biological and echographic markers of cardiac function, we further show a positive and significant impact of the NBD strategy on the changes in BNP levels and cardiac parameters of diastolic dysfunction after 12 weeks of treatment. Importantly, in the multivariable analysis, the effect of NBD remained significant after adjustment for multiple factors, notably BP reduction and changes in renal function. In addition, while the RASB therapeutic strategy was also associated with BP reduction, we observed opposite effects on changes in BNP levels and echocardiographic parameters of diastolic function. This result suggests that an aggressive strategy to reduce sodium balance in patients with RHTN is preferable to rapidly reduce cardiac wall stress and improve cardiac relaxation.

RTHN combines several pathophysiological mechanisms including increased peripheral resistance, fluid retention, and salt sensitivity.[6] RHTN is considered as major risk factor for diastolic dysfunction, a setting in which impaired relaxation is associated with an increase in telediastolic pressure, indirectly reflected by higher levels of BNP.[17,18] As compared with RASB, NDB was associated with a greater reduction in blood volume as indicated by larger reduction in systemic vascular resistance and in GFR and higher increase in renin levels as compared with RASB. However, our results indicate that the improvement in cardiac function with NBD is likely explained by additional effects beyond the plasma volume reduction. The specific pathophysiological cascade bridging RHTN to myocardial dysfunction is imperfectly understood, but previous studies have suggested a stronger link between late systolic load (i.e. arterial stiffness) and early diastolic velocity (E') as measured by tissue doppler imaging.[13] Arterial stiffness was directly assessed in our study by two parameters (PP and PWV[19]), and we interestingly observed that NBD but not RASB induced a significant decrease in PP. Changes over 12 weeks of treatment in PP as well as in PWV were significantly associated with changes in BNP levels in the multivariable analysis, indicating that a greater reduction in arterial stiffness was associated with a better evolution of cardiac diastolic function. Increased PP is an independent factor of cardiovascular risk and outcomes and HF.[20] Reciprocally, decreased central PP results in improved ventricular arterial coupling and cardiac relaxation.[19] A link between salt overload and increased arterial thickness has been well established experimentally and is independent of BP.[21] Arteries play an important role in adapting to an acute sodium load by endothelium-dependent and endothelium-independent mechanisms.[22,23] The transfer of a proportion of the sodium load to the arterial glycocalyx involves the transport of NaCl via the epithelial endothelial

sodium channel (EnNaC).[22,23] In a mouse model of chronic sodium loading, it was shown that the consequent increase in arterial stiffness could be partially prevented by inhibition of EnNaC by spironolactone or amiloride.[24] In our study, it is possible that modamide and spironolactone have induced a decrease in arterial stiffness (thus increasing compliance) leading to a greater decrease in the PP in the NBD group.

On the other hand, the achievement of a complete RASB with ARB, ACEI, and beta-blocker resulted in a significant increase of BNP levels and echographic markers of diastolic function. In the multivariable analysis, the changes in HR were significantly associated with the changes in BNP levels with lower HRs being associated with higher BNP levels. This suggests that an important part of our observation could be explained by a direct detrimental effect of beta-blockers. Beta-blockers and bisoprolol have, however, been reported to exert a positive lusitropic effect in different animal models, an observation that potentially does not translate into humans.[25] In line with our results, it was shown in a substudy of the ASCOT clinical trial that hypertensive patients treated with atenolol displayed higher LV filling pressure, E/E′ and BNP levels as compared with patients receiving amlodipine while the on-treatment BP was similar in both groups.[26] In another study, bisoprolol improved echographic parameters of diastolic function after 3 months of treatment, but these patients did not have RHTN as in our study.[27]

As RHTN is a major risk factor for HF, notably with HFpEF, our results can also be interpreted in the context of the different clinical studies that evaluated therapeutic strategies in patients with HFpEF. Indeed, there is currently no evidence to support a therapeutic benefit for the use of beta-blockers, ACEi, and ARB in patients with HFpEF.[28] Furthermore, these results are in line with the ONTARGET study, which did not show any benefit of a double RAS blockade in terms of cardiovascular events or hospitalization for HF but on the contrary an increased risk of hypotension and renal failure.[29] Reciprocally, the use of spironolactone is likely associated with a reduction in HF rehospitalization in HFpEF patients, but the effects on mortality and quality of life remain unclear.[28,30] Our results further suggest that spironolactone, in combination with other diuretics, might be beneficial to prevent further development of HF, notably with preserved ejection fraction. This observation would however deserve further long-term studies.

Study limitations

The studied population has a major risk factor of HF, but none had a history of HF, so the results cannot be extrapolated to HFpEF patients. BNP and echocardiography were only available at week 0 and week 12 and in a fraction of the patients, so we could not analyse the effect of each titration of the treatment on these parameters. Moreover, some more recent echocardiographic parameters such as left atrial volume and LV longitudinal strain were not measured in our study. The scores to estimate cardiac diastolic dysfunction were derived using left atrial areas rather than volumes as lastly recommended. However, similar trends with improvement in the NBD group were also observed after deriving the scores without considering LA areas (data not shown), thus suggesting that using LA areas instead of LA volumes was not changing our overall results.

In conclusion, our data suggest that combined diuretic treatment (NBD strategy) that targets fluid retention improves diastolic dysfunction and decreases arterial stiffness. Reciprocally, our results suggest that a more complete inhibition of the renin-angiotensin system does not improve diastolic function. These differences involve mechanisms associated with BP reduction and improved ventricular arterial coupling. An intensive strategy with a combination of diuretics should be preferably considered in patients presenting RHTN.

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Conflict of interest

None declared.

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References

1. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GMC, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J* 2016; **37**: 2129–2200.
2. Palmiero P, Zito A, Maiello M, Cameli M, Modesti PA, Muiesan ML, Novo S, Saba PS, Scicchitano P, Pedrinelli R, Ciccone MM. Left ventricular diastolic function in hypertension: methodological

considerations and clinical implications. *J Clin Med Res* 2015; **7**: 137–144.
3. WilliamsB, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, Clement DL, Coca A, de Simone G, Dominiczak A, Kahan T, Mahfoud F, Redon J, Ruilope L, Zanchetti A, Kerins M, Kjeldsen SE, Kreutz R, Laurent S, Lip GYH, McManus R, Narkiewicz K, Ruschitzka F, Schmieder RE, Shlyakhto E, Tsioufis C, Aboyans V, Desormais I, De Backer G, Heagerty AM, Agewall S, Bochud M, Borghi C, Boutouyrie P, Brguljan J, Bueno H, Caiani EG, Carlberg B, Chapman N, Cífková R, Cleland JGF, Collet J-P, Coman IM, de Leeuw PW, Delgado V, Dendale P, Diener H-C, Dorobantu M, Fagard R, Farsang C, Ferrini M, Graham IM, Grassi G, Haller H, Hobbs FDR, Jelakovic B, Jennings C, Katus HA, Kroon AA, Leclercq C, Lovic D, Lurbe E, Manolis AJ, McDonagh TA, Messerli F, Muiesan ML, Nixdorff U, Olsen MH, Parati G, Perk J, Piepoli MF, Polonia J, Ponikowski P, Richter D J, Rimoldi SF, Roffi M, Sattar N, Seferovic PM, Simpson IA, Sousa-Uva M, Stanton AV, van de Borne P, Vardas P, Volpe M, Wassmann S, Windecker S, Zamorano JL, Windecker S, Aboyans V, Agewall S, Barbato E, Bueno H, Coca A, Collet J-P, Coman IM, Dean V, Delgado V, Fitzsimons D, Gaemperli O, Hindricks G, Iung B, Jüni P, Katus HA, Knuuti J, Lancellotti P, Leclercq C, McDonagh TA, Piepoli MF, Ponikowski P, Richter DJ, Roffi M, Shlyakhto E, Simpson IA, Sousa-Uva M, Zamorano JL, Tsioufis C, Lurbe E, Kreutz R, Bochud M, Rosei EA, Jelakovic B, Azizi M, Januszewics A, Kahan T, Polonia J, van de Borne P, Williams B, Borghi C, Mancia G, Parati G, Clement DL, Coca A, Manolis A, Lovic D, Benkhedda S, Zelveian P, Siostrzonek P, Najafov R, Pavlova O, De Pauw M, Dizdarevic-Hudic L, Raev D, Karpettas N, Linhart A, Olsen MH, Shaker AF, Viigimaa M, Metsärinne K, Vavlukis M, Halimi J-M, Pagava Z, Schunkert H, Thomopoulos C, Páll D, Andersen K, Shechter M, Mercuro G, Bajraktari G, Romanova T, Trušinskis K, Saade GA, Sakalyte G, Noppe S, DeMarco DC, Caraus A, Wittekoek J, Aksnes TA, Jankowski P, Polonia J, Vinereanu D, Baranova EI, Foscoli M, Dikic AD, Filipova S, Fras Z, Bertomeu-Martínez V, Carlberg B, Burkard T, Sdiri W, Aydogdu S, Sirenko Y, Brady A, Weber T, Lazareva I, Backer TD, Sokolovic S, Jelakovic B, Widimsky J, Viigimaa M, Pörsti I, Denolle T, Krämer BK, Stergiou GS, Parati G, Trušinskis K, Miglinas M, Gerdts E, Tykarski A, de Carvalho Rodrigues M, Dorobantu M, Chazova I, Lovic D, Filipova S, Brguljan J, Segura J, Gottsäter A, Pechère-Bertschi A, Erdine S, Sirenko Y, Brady A,. 2018 ESH Guidelines for th/ ESH Guidelines for the management of arterial hypertension. *European Heart Journal*. 2018;**39**: 33:3021–3104. https://doi.org/10.1093/eurheartj/ehy339
4. Daugherty SL, Powers JD, Magid DJ, Tavel HM, Masoudi FA, Margolis KL, O'Connor PJ, Selby JV, Ho PM. Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation* 2012; **125**: 1635–1642.
5. Gaddam KK, Nishizaka MK, Pratt-Ubunama MN, Pimenta E, Aban I, Oparil S, Calhoun DA. Characterization of resistant hypertension: association between resistant hypertension, aldosterone, and persistent intravascular volume expansion. *Arch Intern Med* 2008; **168**: 1159–1164.
6. Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, White A, Cushman WC, White W, Sica D, Ferdinand K, Giles TD, Falkner B, Carey RM, American Heart Association Professional Education Committee. Resistant hypertension: diagnosis, evaluation, and treatment. *Circulation* 2008; **117**: e510–e526.
7. Ferrario CM, Schiffrin EL. Role of mineralocorticoid receptor antagonists in cardiovascular disease. *Circ Res* 2015; **116**: 206–213.
8. Flatt DM, Brown MC, Mizeracki AM, King BJ, Weber KT. Mineralocorticoid receptor antagonists in the management of heart failure and resistant hypertension: a review. *JAMA Cardiol* 2016; **1**: 607–612.
9. Tsujimoto T, Kajio H. Beta-blocker use and cardiovascular event risk in patients with heart failure with preserved ejection fraction. *Sci Rep* 2018; **8**: 9556.
10. Bobrie G, Frank M, Azizi M, Chatellier G. Sequential nephron blockade versus sequential renin-angiotensin system blockade in resistant hypertension: a prospective, randomized, open blinded endpoint study. *J Hypertens* 2012; **30**: 1656–1664.
11. Azizi M, Perdrix L, Bobrie G, Frank M, Chatellier G, Ménard J, Plouin PF. Greater efficacy of aldosterone blockade and diuretic reinforcement vs dual renin–angiotensin blockade for left ventricular mass regression in patients with resistant hypertension. *J Hypertens* 2014; **32**: 2038–2044.
12. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ, Chamber Quantification Writing Group, American Society of Echocardiography's Guidelines and Standards Committee, European Association of Echocardiography. Recommendations for chamber quantification. *J Am Soc Echocardiogr* 2005; **18**: 1440–1463.
13. NaguehSF, Smiseth OA, Appleton CP, Byrd BF, Dokainish H, Edvardsen T, Flachskampf FA, Gillebert TC, Klein AL, Lancellotti P, Marino P, Oh JK, Popescu BA, Waggoner AD. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Journal of the American Society of Echocardiography*. 2016;**29**: 4:277–314. https://doi.org/10.1016/j.echo.2016.01.011
14. Skimming JW, Cassin S, Nichols WW. Calculating vascular resistances. *Clin Cardiol* 1997; **20**: 805–808.
15. The Reference Values for Arterial Stiffness' Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur Heart J* 2010; **31**: 2338–2350.
16. Bortel LV, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, Backer TD, Filipovsky J, Huybrechts S, Mattace-Raso FUS, Protogerou AD, Schillaci G, Segers P, Vermeersch S, Weber T, Society A, European Society of Hypertension Working Group on Vascular Structure and Function, European Network for Noninvasive Investigation of Large Arteries. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens* 2012; **30**: 445–448.
17. Aeschbacher B. Diastolic dysfunction precedes myocardial hypertrophy in the development of hypertension. *Am J Hypertens* 2001; **14**: 106–113.
18. Brandt MC, Mahfoud F, Reda S, Schirmer SH, Erdmann E, Böhm M, Hoppe UC. Renal sympathetic denervation reduces left ventricular hypertrophy and improves cardiac function in patients with resistant hypertension. *J Am Coll Cardiol* 2012; **59**: 901–909.
19. Ikonomidis I, Aboyans V, Blacher J, Brodmann M, Brutsaert DL, Chirinos JA, de Carlo M, Delgado V, Lancellotti P, Lekakis J, Mohty D, Nihoyannopoulos P, Parissis J, Rizzoni D, Ruschitzka F, Seferovic P, Stabile E, Tousoulis D, Vinereanu D, Vlachopoulos C, Vlastos D, Xaplanteris P, Zimlichman R, Metra M. The role of ventricular–arterial coupling in cardiac disease and heart failure: assessment, clinical implications and therapeutic interventions. *Eur J Heart Fail* 2019; **21**: 402–424.
20. Selvaraj S, Steg PG, Elbez Y, Sorbets E, Feldman LJ, Eagle KA, Ohman EM, Blacher J, Bhatt DL, REACH Registry Investigators. Pulse pressure and risk for cardiovascular events in patients with atherothrombosis: From the REACH registry. *J Am Coll Cardiol* 2016; **67**: 392–403.
21. Edwards D, Farquhar W. Vascular effects of dietary salt. *Curr Opin Nephrol Hypertens* 2015; **24**: 8–13.
22. Oberleithner H. Vascular endothelium: a vulnerable transit zone for merciless sodium. *Nephrol Dial Transplant* 2014; **29**: 240–246.
23. Judd EK, Calhoun DA, Warnock DG. Pathophysiology and treatment of resistant hypertension: the role of aldosterone and amiloride-sensitive sodium

channels. *Semin Nephrol* 2014; **34**: 532–539.
24. Paar M, Pavenstädt H, Kusche-Vihrog K, Drüppel V, Oberleithner H, Kliche K. Endothelial sodium channels trigger endothelial salt sensitivity with aging. *Hypertension* 2014; **64**: 391–396.
25. Ma L, Gul R, Habibi J, Yang M, Pulakat L, Whaley-Connell A, Ferrario CM, Sowers JR. Nebivolol improves diastolic dysfunction and myocardial remodeling through reductions in oxidative stress in the transgenic (mRen2) rat. *Am J Physiol-Heart Circ Physiol* 2012; **302**: H2341–H2351.
26. Tapp RJ, Sharp A, Stanton AV, O'Brien E, Chaturvedi N, Poulter NR, Sever PS, Thom SAMG, Hughes AD, Mayet J. Differential effects of antihypertensive treatment on left ventricular diastolic function. *J Am Coll Cardiol* 2010; **55**: 1875–1881.
27. Ilgenli TF, Kilicaslan F, Kirilmaz A, Uzun M. Bisoprolol improves echocardiographic parameters of left ventricular diastolic function in patients with systemic hypertension. *Cardiology* 2006; **106**: 127–131.
28. Martin N, Manoharan K, Thomas J, Davies C, Lumbers RT. Beta-blockers and inhibitors of the renin-angiotensin aldosterone system for chronic heart failure with preserved ejection fraction. Cochrane Heart Group, editor. *Cochrane Database Syst Rev* 2018: CD012721.
29. ONTARGET Investigators, Yusuf S, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P, Anderson C. Telmisartan, ramipril, or both in patients at high risk for vascular events. *N Engl J Med* 2008; **358**: 1547–1559.
30. Pitt B, Pfeffer MA, Assmann SF, Boineau R, Anand IS, Claggett B, Clausell N, Desai AS, Diaz R, Fleg JL, Gordeev I, Harty B, Heitner JF, Kenwood CT, Lewis EF, O'Meara E, Probstfield JL, Shaburishvili T, Shah SJ, Solomon SD, Sweitzer NK, Yang S, McKinlay SM. Spironolactone for heart failure with preserved ejection fraction. *N Engl J Med* 2014; **370**: 1383–1392.