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Potential Spironolactone effects on collagen metabolism biomarkers in patients with uncontrolled blood pressure

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Abstract

Background: An increase in myocardial collagen content may contribute to the development of heart failure; this might be inhibited or reversed by mineralocorticoid receptor antagonists (MRAs). We investigated changes in serum concentrations of the collagen synthesis biomarkers N-terminal propeptide of procollagen type III (PIIINP) (primary outcome) and C-terminal propeptide of procollagen type I (PICP) (secondary outcome) after non-randomized initiation of spironolactone as add-on therapy amongst patients with resistant hypertension enrolled in the "Anglo-Scandinavian Cardiac Outcomes" (ASCOT) trial.

Methods: An age/sex matching plus propensity-scored logistic regression model incorporating variables related to the outcome and spironolactone treatment was created to compare patients treated with spironolactone for 9-month periods vs. matched controls. A within-person analysis comparing changes in serum biomarker concentrations in the 9-month before vs. after spironolactone treatment was also performed.

Results: Patients included in the between-person analysis (n=146) were well matched: the mean age was 63±7 years and 11% were woman. Serum concentrations of PIIINP and PICP rose in "controls" and fell during spironolactone treatment (adjusted means +0.52 [-0.05 to 1.09] vs. -0.41 [-0.97 to 0.16] ng/mL, p=0.031 for PIIINP and +4.54 [-1.77 to 10.9] vs. -6.36 [-12.5 to -0.21] ng/mL, p=0.023 for PICP). For the within-person analysis (n=173), spironolactone treatment was also associated with a reduction in PICP (beta estimate = -11.82 [-17.53 to -6.10] ng/mL, p<0.001) but not in PIIINP levels.

Conclusions: Treatment with spironolactone was associated with a reduction in serum biomarkers of collagen synthesis independently of blood pressure in hypertensive patients, suggesting that spironolactone might exert favorable effects on myocardial collagen synthesis and fibrosis. Whether this effect might contribute to slowing the progression to heart failure is worth investigating.

Key-words: resistant hypertension; collagen markers; fibrosis; heart failure; spironolactone; prevention.

Key Messages

What is already known about this subject?

Spironolactone is the most effective add-on drug for the treatment of resistant hypertension.

What does this study add?

From a practical standpoint the present manuscript reinforces the current knowledge as it demonstrates that beyond its blood pressure lowering properties, spironolactone can reduce myocardial fibrosis and by this mechanism potentially delay heart failure onset.

How might this impact on clinical practice?

Spironolactone could be used not only for the lowering of blood pressure in patients with resistant hypertension but also for the reduction of myocardial fibrosis and potentially heart failure.
Whether spironolactone should be added earlier in the treatment of hypertension requires prospective validation.

Abbreviation list:

HF, heart failure

BP, blood pressure

CV, cardiovascular

MRAs, mineralocorticoid receptor antagonists

MI, myocardial infarction

PIIINP, N-terminal propeptide of procollagen type III

PICP, C-terminal propeptide of procollagen type I

CITP, C-terminal telopeptide of collagen type I

MMP-1, matrix-metalloproteinase-1

NT-proBNP, N-terminal pro brain natriuretic peptide

hsTnT, high-sensitivity troponin T

Introduction

Heart failure (HF) is a serious and growing problem that impairs quality of life, causes recurrent hospitalizations and shortens life expectancy[1] and thus greater efforts to delay or prevent its onset are justified[2]. For patients with hypertension, effective blood pressure (BP) control reduces the incidence of cardiovascular (CV) events[3, 4], especially HF[5].

An increase in myocardial and vascular collagen content ("fibrosis") is common in hypertensive patients and may be a major determinant of transition to and progression of HF[6-9]. Mineralocorticoid receptor antagonists (MRAs), such as spironolactone, are a highly effective treatment for resistant hypertension[10] and also reduce plasma/serum biomarkers of collagen synthesis in patients with HF, myocardial infarction, and metabolic syndrome[11-15]. Whether MRAs also reduce collagen synthesis biomarkers in patients with hypertension and whether this is independent of their effect on blood pressure is unknown. If MRAs have such a dual mechanism of action, they could be particularly effective at preventing HF.

Accordingly, we studied the effects of spironolactone on serum collagen metabolism biomarkers in a subset of patients with resistant hypertension that participated in the "Anglo-Scandinavian Cardiac Outcomes" (ASCOT) trial[16].

Methods

Trial design

The design, patient eligibility criteria, study procedure and main results of the Anglo-Scandinavian Cardiac Outcomes trial-blood pressure lowering arm (ASCOT-BPLA) have been previously reported[17]. In short, the ASCOT-BPLA was a multicentre, prospective, randomised controlled trial, enrolling 19,257 patients with hypertension who were aged 40–79 years and had at least three other cardiovascular risk factors. Patients were assigned either amlodipine adding perindopril as required (n=9,639) or atenolol adding bendroflumethiazide and potassium as required (n=9,618). Spironolactone, as a fourth-line agent for resistant hypertension, was evaluated in 1,411 participants as add-on therapy prescribed in a non-randomized fashion at the discretion of the treating physician[16]. The median duration of spironolactone treatment was 1.3 years (interquartile range: 0.6 to 2.6 years) and the median dose of spironolactone was 25 mg (interquartile range: 25 to 50 mg) at both the start and end of the observation period. Spironolactone reduced mean blood pressure by 22/10 mmHg independently of age, sex, smoking, and diabetic status. Only patients treated with spironolactone for at least 9 months and with available serum samples were selected for this observational analysis, as it was thought that short-term intervention might have little or no effect on collagen turnover (please see also the methods section). Further patient selection for this analysis is shown in **Figure 1**.

Ethical approval and signed informed consent were required to participate in the ASCOT trial.

Study aims and biomarker assessment

The main aims of this analysis were to compare changes in serum concentrations of N-terminal propeptide of procollagen type III (PIIINP) – *primary outcome measure* and C-terminal propeptide of procollagen type I (PICP) – *secondary outcome measure* plus C-terminal telopeptide of collagen type I (CITP), matrix-metalloproteinase-1 (MMP-1), CITP/MMP-1 ratio, and PIIINP/CITP ratio – *exploratory measures* in spironolactone-treated patients vs. matched controls (between-person analysis). Additionally, a within-person analysis was performed by assessing the changes in the biomarker levels in spironolactone-treated patients (spironolactone period) compared to the 9 months prior to spironolactone treatment (control period) using the same outcome measures as above described. The use of PIIINP as primary outcome measure was chosen for testing the primary hypothesis of the HOMAGE ("Heart 'omics' in AGEing") trial (NCT02556450) in which patients at high-risk for developing HF are randomized to either spironolactone plus conventional therapy or conventional therapy alone to assess the effect of spironolactone on PIIINP changes from baseline to 9 months. The assessment of PICP changes as secondary outcome measure was based on the increasing body of evidence supporting the direct correlation of this biomarker with myocardial fibrosis[18]. The evidence supporting the correlation of the other studied biomarkers with myocardial fibrosis is weaker and they were assessed as exploratory measures.

The rationale for the use of the above referenced "ratios" is as follows: the CITP/MMP-1 ratio has been shown to be inversely correlated with myocardial collagen cross-linking in HF patients[7]. As collagen cross-linking determines the resistance of the collagen fiber to MMP degradation, the higher the cross-linking of collagen type I fibers, the lower the cleavage of the cross-linked peptide CITP by the enzyme MMP-1. The PIIINP/CITP ratio has been found to be associated with higher event-rate in patients with MI and it has been used as a way to evaluate the collagen turnover as it is a ratio between a synthesis and a degradation marker[19].

Changes in serum N-terminal pro brain natriuretic peptide (NT-proBNP) and high sensitive troponin T (hsTnT), were also assessed as exploratory analyses.

The 9-month assessment visit was chosen based on the observation that in more "severe" and symptomatic populations (such as RALES and EPHESUS: HF-REF with severe symptoms and MI with systolic dysfunction, respectively) a lowering in collagen markers in patients randomized to MRA therapy was observed at 6 months[12, 13], hence we hypothesize that less "severe" patients (such as those included in ASCOT and HOMAGE) spironolactone may require more time to demonstrate its "anti-fibrotic" effects.

Blood samples were drawn at 9-month before spironolactone treatment (visit 1, V1), baseline (visit 2, V2; the first day of spironolactone treatment), and after 9-month of spironolactone treatment (visit 3, V3). All samples were centrifuged immediately at 3000 rpm for 10 minutes and stored at -

80°C until assay analysis. Samples were available for at least two time-points. All samples were transported to the central laboratory and assayed in 1 batch. All assays were performed by technicians blinded to clinical data and subject randomization.

A commercial radioimmunoassay (Orion Diagnostica) was used to measure PIIINP. The lower limit of detection was 0.3 µg/L. Serum PICP was measured by using the METRA EIA kit (Quidel Corporation). The lower limit of detection was 0.2 µg/L. Inter-assay variability was <12% and intra-assay variations was <10% for both. Serum NT-proBNP was measured using an ELISA method (Roche Diagnostics). The inter-assay and intra-assay coefficients of variation were less than 7% and the lower limit of detection was 5 pg/mL. Serum hsTnT was measured with a highly sensitive assay (Troponin T hs STAT, Roche Diagnostics). The lower detection limit of the assay was 0.005 g/L and the inter-assay coefficient of variation was 4.7%. Serum CITP was measured by ELISA (Orion Diagnostica). The inter-assay and intra-assay coefficients of variation were 9.4% and 11.2%. The lower limit of detection was 0.3 µg of CITP per liter. Total serum MMP-1 was measured by an ELISA method (GE Healthcare). The inter-assay and intra-assay coefficients of variation were 11.6% and 5.5%, respectively and the lower limit of detection was 1.7 µg/L.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation (SD) and median (percentile $_{25\text{-}75}$). Categorical variables are presented was absolute numbers (n.) and frequencies (%). The studied biomarkers had a skewed distribution, however their "delta" (9-month value – baseline value) had normal distribution. Comparisons of patients` characteristics were performed using paired t-test, Wilcoxon signed-rank test or McNemar's test as appropriate. Two analysis strategies were applied: 1) between-person analysis (i.e. spironolactone treated vs. matched controls) with 73 matched pairs identified. **Figure 1**; and 2) within-person analysis (i.e. comparison biomarker changes in the 9-month previous to spironolactone treatment [control period] vs. the 9-month after spironolactone treatment [spironolactone period]), with a total of 173 patients fulfilling this pattern. **Figure 1**.

For the between-person analysis, spironolactone-treated vs. control patients were matched on age, sex and time since study participation. As the ASCOT study was not randomized according to spironolactone treatment, differences between spironolactone-treated patients and matched controls could still occur. In order to address this issue, we created a propensity score based on a logistic regression model that incorporated all variables independently associated both with the studied outcomes and the treatment decision. Smoking status, body mass index, systolic blood pressure, diastolic blood pressure, heart rate, total cholesterol, diabetes, study drug (amlodipine/atenolol) and initial value of NT-proBNP were used to compute the propensity score (an alternative propensity score was computed without baseline NT-proBNP for analyses evaluating the change in NT-proBNP). The generated propensity score was then used as adjustment variable. General linear models were performed to assess the association between spironolactone treatment and the change in biomarker

levels. For the within-person comparisons (control period vs. spironolactone period), each subject had 3 biomarker values, allowing the computation of biomarker change in the 9 months before and after spironolactone treatment. Mixed models (repeated measures) were then used to assess biomarker changes. As the changes in biomarker levels may depend on the initial value of the biomarker, all analyses were adjusted on the biomarker initial value (i.e. the value of the biomarker at V1 when assessing the change between V1 and V2, and value of the biomarker at V2 when assessing the change between V2 and V3) plus the propensity score. For the between-person comparisons, each subject had only 1 value of biomarker change. Linear regression models were computed in this case, adjusted on the initial value of the biomarker plus the propensity score (as well as both variables age and gender which were involved in the matching) and an additional model was built with adjustment on systolic blood pressure changes. In the presence of outliers, the outcome values below the 5^{th} and above the 95^{th} percentile were excluded (we also performed the same set of analyses in the whole population i.e. including outliers, with overlapping results; data not shown). Results are expressed as beta estimates and respective 95% confidence intervals. A p-value of <0.05 was considered statistically significant. All analyses were performed using software SAS version 9.4 (SAS Institute Inc., Cary, N.C., USA).

Study flow-chart

A total of 252 patients were selected based on their pattern of spironolactone treatment (i.e., at least 9-month of treatment plus available blood samples). For the analysis we required samples for at least two time-points (i.e. V2 + V3) for the between-person matched analysis, and at least three (i.e. V1 + V2 + V3) for the within-person analysis. This left 146 patients for the between- person analysis (73 "spironolactone-treated" vs. 73 "controls"), and 173 patients for the within-person analysis. Sixty-seven patients had features allowing their incorporation in both between- and within-person analysis. **Figure 2**.

Results

Between-person analysis

Patients` characteristics

A total of 146 (73 "cases" and 73 "controls) patients were included in the between-person analysis (matched on age, sex, and study participation time). The mean age was 63±7 years, and the great majority (89%) were men. Most baseline characteristics were similar, but patients initiated on spironolactone had higher systolic blood pressure (167±16 vs. 161±18 years), more often had diabetes (45.2% vs. 26.0%) and were more likely to have been assigned to atenolol (69.9% vs. 42.5%) rather than amlodipine (30.1% vs. 57.5%). **Table 1**.

Biomarker change

Serum concentrations of the collagen synthesis biomarkers PIIINP and PICP fell in spironolactone-treated patients but rose in matched controls (adjusted means of PIIINP change =0.52

[-0.05 to 1.09] for control vs. -0.41 [-0.97 to 0.16] for spironolactone, p=0.031 and adjusted means of PICP change =4.54 [-1.77 to 10.9] for control vs. -6.36 [-12.5 to -0.21] for spironolactone, p=0.023). Changes of borderline statistical significance were observed for the collagen degradation biomarker CITP (adjusted means =-1.19 [-2.06 to -0.32] for control vs. -0.03 [-0.88 to 0.81] for spironolactone, p=0.080). Accordingly, the collagen turnover index (PIIINP/CITP) suggested higher turnover on spironolactone (adjusted means =0.38 [0.14 to 0.63] for control vs. -0.01 [-0.24 to 0.22] for spironolactone, p=0.042). No significant changes in MMP1, CITP/MMP1 ratio, NT-proBNP, and hsTnT were observed. **Table 2** and **Figure 3**. The absolute (i.e. non-adjusted) changes are presented in **Supplemental Table 1**. The additional models adjusted on systolic blood pressure changes (i.e. V3 – V2) are shown in **Supplemental Table 3**. This resulted in a non-significant indirect effect of spironolactone induced by BP changes on the outcomes (p >0.10 for each biomarker, data not shown).

Within-person analysis

Patients` characteristics

The 173 patients included in the within-person (i.e. comparison of the same individuals before and after spironolactone treatment) analysis were older (mean age =64±8 years) and more often women (19.7%) than the matched case-controls but serum biomarker concentrations were similar. **Table 1**.

Biomarker change

Periods of treatment with spironolactone (compared to the period without treatment) were associated with a serum PICP fall (adjusted means =3.63 [0.08 to 7.18] before spironolactone vs. -8.20 [-11.7 to -4.7] on spironolactone, p<0.001). No significant changes were observed regarding the other collagen biomarkers. Serum NT-proBNP fell during spironolactone treatment (adjusted means = 33 [16 to 50] for the period without spironolactone vs. -21 [-39 to -3] on spironolactone, p<0.001). **Table 2**. The absolute (i.e. non-adjusted) changes are presented in the **Supplemental Table 2**. and the adjusted biomarker changes incorporating also the delta systolic blood pressure at the time of biomarker measurements (i.e. V2 – V1 and V3 – V2) showed similar results to those presented in Table 2 (data not shown).

Discussion

This analysis suggests that treating patients with resistant hypertension and additional risk factors with spironolactone may be associated with a fall in serum concentrations of PIIINP and PICP, markers of collagen synthesis, and an increase in CITP a marker of collagen degradation, which might reflect a favourable effect on extracellular matrix remodelling and myocardial fibrosis. These changes were independent from the effects of spironolactone on blood pressure. We speculate that these favourable effects on extracellular matrix remodelling in patients at high risk might translate into clinically meaningful benefits by slowing the transition to LV diastolic dysfunction, atrial and/or

ventricular arrhythmias and, ultimately, HF[6, 20].

Prolonged myocardial stress due to hypertension and other risk factors is thought to increase extracellular matrix (ECM) deposition, leading to fibrosis that may compromise myocardial function and impair electrical conduction favoring the advent of arrhythmias and HF[6, 20, 21]. Collagen synthesis is a dynamic process involving metabolically active myofibroblasts[20]. In this regard, PIIINP is released into the bloodstream after cleavage from procollagen type III[22]. Serum PIIINP correlates with myocardial collagen type III in HF patients of ischemic etiology and idiopathic dilated cardiomyopathy (DCM), and higher concentrations are associated with a worse prognosis[23, 24]. The evidence supporting the effect of spironolactone in reducing PIIINP levels in humans with systolic dysfunction is robust. In patients with DCM the reduction of the myocardial collagen (as assessed by left ventricular endomyocardial biopsy) after treatment with spironolactone was accompanied by a reduction in serum PIIINP concentrations[25]. In 261 HF patients with reduced left ventricular ejection fraction and severe symptoms enrolled in the Randomized Aldactone Evaluation Study (RALES), serum concentrations of PIIINP above median (>3.9 ng/mL) were associated with higher mortality rates (HR; 95%CI =2.36; 1.34-4.18) and serum PIIINP decreased in spironolactone treated patients from baseline to 6 months but not in those assigned to placebo[12]. In MI patients with systolic dysfunction and/or HF enrolled in the Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS)[13], eplerenone also reduced serum PIIINP. In 134 patients with acute anterior ST elevation MI (STEMI), intravenous potassium canrenoate (the active metabolite of spironolactone) also reduced serum PIIINP[26]. More recently, the REMINDER trial assessed the effect of eplerenone initiated within 24 h of symptom-onset in patients with an acute STEMI without known HF[27]. In a subanalysis including 526 patients with collagen biomarkers measurements, only those with PIIINP levels above the median of 3.9 ng/mL had a significant reduction of this biomarker by eplerenone (as compared to placebo)[11]. The median baseline levels of PIIINP in the ASCOT trial (median =5 ng/mL, percentile$_{25\text{-}75}$ =4-6 ng/mL) were similar to those reported in the REMINDER (=4 ng/mL), EPHESUS (=4 ng/mL)[13] and RALES (=4 ng/mL)[12] trials, and lower than those reported for patients in a study of DCM (=6 ng/mL)[23]. Suggesting that collagen turnover may be similar across a range of cardiovascular diseases.

Serum PICP levels are highly correlated with total myocardial collagen volume fraction (assessed in myocardial samples with collagen-specific staining) in patients with hypertension and HF[28, 29]. However, the effect of MRA on serum concentrations of PICP have been less reproducible and of smaller magnitude as compared to the effect of MRAs on PIIINP. In RALES, PICP levels were not significantly reduced by spironolactone[12]. In 80 patients with metabolic syndrome spironolactone (vs. placebo) decreased circulating PICP levels (and also PIIINP), and PICP change correlated with improvement in left ventricular systolic function assessed by echocardiographic strain[14]. In 113 patients with obesity (body mass index $\geq$30 Kg/m^2) without other comorbidities, spironolactone (vs. placebo) reduced serum PICP as well as PIIINP; change in PICP (but not PIIINP) was associated with

improvement in left ventricular diastolic function[15]. However, these findings were not reproduced in patients with diabetic cardiomyopathy[30] (and PICP was not available in the EPHESUS and REMINDER trials). Both in the between- and within-person analysis marked effects on the drop of PICP levels were observed. PICP originates during the conversion of procollagen type I to collagen type I in a 1:1 ratio, hence serum PICP concentrations are direct indicators of collagen synthesis[22].

CITP is cleaved by the action of MMP-1 on collagen type I fibers and may reflect collagen type I degradation, however its association with myocardial fibrosis is not well established[22]. The CITP/MMP-1 ratio did not significantly change with spironolactone treatment, suggesting that spironolactone did not affect collagen cross-linking in the present analysis[7]. The PIIINP (collagen type III synthesis) to CITP (collagen type I degradation) ratio may serve as an indirect marker of collagen turnover[19]. Spironolactone may have had a beneficial effect on collagen turnover (i.e. less synthesis and more degradation) in this analysis.

NT-proBNP fell with spironolactone in the within-person analysis. This may reflect a reduction in myocardial stress due to the reduction in blood pressure, a contraction in blood volume due to natriuresis, improved myocardial function due potassium retention as well as effects on collagen metabolism. The failure to observe an effect of spironolactone in the between-patient analysis may reflect the greater heterogeneity in NT-proBNP between patients. Serum concentrations of troponin were low and did not change in either analysis.

The ongoing HOMAGE trial (NCT02556450) is investigating whether spironolactone (compared to "control") can favorably alter extra-cellular matrix remodeling, assessed by changes in circulating PIIINP (primary outcome), PICP, NT-proBNP and echocardiographic measures from randomization to 9 months, in patients at increased risk of developing HF[2, 31]. This analysis provides some preliminary evidence to support the HOMAGE hypothesis. However, the widespread use of MRAs for the prevention of HF cannot be recommended until adequately powered studies demonstrate clinical benefits. Targeting patients with elevated serum concentrations of PIIINP and PICP indicating an active "pro-fibrotic" profile may increase efficacy and avoid a potentially hazardous treatment for patients who have little to gain.

Clinical implications

Spironolactone is the most effective add-on drug for the treatment of resistant hypertension[10]. From a practical standpoint the present manuscript reinforces the current knowledge as it demonstrates that beyond its blood pressure lowering properties, spironolactone can reduce myocardial fibrosis and by this mechanism potentially delay HF onset. Therefore, spironolactone could be used not only for the lowering of blood pressure in patients with resistant hypertension but also for the reduction of myocardial fibrosis and potentially HF. Whether spironolactone should be added earlier in the treatment of hypertension requires prospective validation.

Limitations

Several limitations should be acknowledged in this analysis. This is a post-hoc study and the treatment of interest was not randomized; hence caution should be exercised in inferring any causal relationship and all the limitations inherent to observational studies are also applied herein. However, the study adds to a growing body of, as yet, inconclusive evidence. The propensity score technique cannot include unmeasured potential confounders. The between-person analysis also carries important confounders such as treatment effects and events that change over time within the same individual and that cannot be estimated separately. These findings lack external validation and should be prospectively confirmed in other cohorts (as in the ongoing HOMAGE program). Internal validation also showed caveats as PIIINP fell in patients treated with spironolactone in the between-person analysis but not in the within-person analysis. This may be due to bias and limitations inherent to these two approaches. Moreover, no imaging evaluation was available; hence we cannot ascertain if the changes in the collagen turnover biomarkers was accompanied by an improvement in cardiac structure and function. As the biomarker measurements were performed at only two or three time-points in order to evaluate our hypothesis, no kinetic of the effect of spironolactone could be assessed, therefore we cannot ascertain whether these changes were present before the 9-month measurement. Echocardiography was not routinely performed in the ASCOT trial; hence this information was not available for the present analysis. Echocardiographic variables could have provided further insight on whether these collagen marker changes were actually accompanied by improvements in the heart structure and function. Finally, we do not know how large a change in collagen turnover biomarkers is clinically relevant.

Conclusions

Spironolactone, independently of blood pressure changes, was associated with a reduction in serum collagen synthesis biomarkers in patients with resistant hypertension, suggesting a potential beneficial effect of spironolactone on the cardiac extracellular matrix of this population at high-risk of developing HF. Further randomized trials are needed to properly assess this potential and, if so, whether such changes translate to clinical benefits to prevent new onset HF.

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Disclosures

None.

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Table 1. Characteristics of the patients in the between-person ("spironolactone" / "control") and within-person analysis

Patients` characteristics	Between-person					Within-person	
	Matched control (N=73)		Spironolactone (N=73)				
	N		N		p*	N	
Age, years	73	63 ± 7	73	63 ± 7	-	173	64 ± 8
Women, n (%)	73	8 (11.0%)	73	8 (11.0%)	-	173	34 (19.7%)
Smoking status, n (%)	73	-	73	-	0.28	173	-
Current smoker	-	16 (21.9%)	-	19 (26.0%)	-	-	31 (17.9%)
Past smoker	-	39 (53.4%)	-	30 (41.1%)	-	-	73 (42.2%)
Body mass index, kg/m²	73	28.7 ± 4.5	73	30.0 ± 4.1	0.081	173	30.1 ± 4.4
SBP, mmHg	73	161 ± 16	73	167 ± 18	0.035	173	167 ± 19
DBP, mmHg	73	93 ± 9	73	93 ± 11	0.91	173	92 ± 11
Heart rate, bpm	73	72 ± 11	73	74 ± 15	0.23	173	71 ± 14
Cholesterol, mmol/L	73	5.8 ± 1.1	73	5.8 ± 1.1	0.82	173	5.8 ± 1.1
LDL, mmol/L	69	3.7 ± 1.1	69	3.6 ± 1.0	0.41	163	3.7 ± 1.0
eGFR, mL/min/1.73m²	45	72 (61 - 79)	50	72 (62 - 78)	0.46	121	70 (62 - 77)
Potassium, mmol/L	67	4.2 ± 0.4	73	4.3 ± 0.5	0.56	165	4.2 ± 0.6
Blood glucose, mmol/L	70	5.7 (5.2 - 6.7)	70	6.1 (5.3 - 7.8)	0.31	166	6.0 (5.2 - 7.9)
Diabetes, n (%)	73	19 (26.0%)	73	33 (45.2%)	0.020	173	80 (46.2%)
Study drug, n (%)	73	-	73	-	0.0009	173	-
Amlodipine	-	42 (57.5%)	-	22 (30.1%)	-	-	53 (30.6%)
Atenolol	-	31 (42.5%)	-	51 (69.9%)	-	-	120 (69.4%)
ACE inhibitor, n (%)	69	25 (36.2%)	70	30 (42.9%)	0.49	168	61 (36.3%)
ARB, n (%)	69	4 (5.8%)	70	5 (7.1%)	1.00	168	10 (6.0%)
Beta blocker, n (%)	69	26 (37.7%)	70	26 (37.1%)	1.00	168	71 (42.3%)
Thiazide diuretics, n (%)	69	28 (40.6%)	70	27 (38.6%)	0.86	168	69 (41.1%)
LVH, n (%)	73	20 (27.4 %)	73	18 (24.7 %)	0.71	173	45 (26.0%)

PIIINP, ng/mL	73	4.5 (3.7 - 6.3)	71	5.1 (3.6 - 6.4)	0.90	171	4.9 (3.7 - 6.3)
PICP, ng/mL	68	76 (63 - 90)	68	80 (66 - 102)	0.076	166	78 (66 - 100)
CITP, ng/mL	68	5.9 (3.3 - 9.1)	68	7.1 (4.4 - 8.9)	0.29	166	6.4 (4.2 - 8.8)
PIIINP/CITP ratio	68	0.90 (0.49 - 1.58)	67	0.73 (0.49 - 1.33)	0.33	165	0.77 (0.52 - 1.33)
MMP1, ng/mL	68	5.5 (4.4 - 9.1)	68	5.9 (5.1 - 10.0)	0.60	166	6.2 (5.0 - 10.2)
CITP/MMP1 ratio	68	2.76 (1.55 - 5.25)	68	3.23 (1.78 - 4.73)	0.44	166	3.16 (1.36 - 4.94)
NT-proBNP, pg/mL	70	95 (48 - 239)	71	168 (66 - 324)	0.051	166	176 (87 - 354)
hsTnT, pg/mL	71	9 (6 - 14)	71	10 (7 - 13)	0.82	166	10 (7 - 14)

* paired t-test for normal variables, wilcoxon signed-rank test for skewed variables, McNemar's test for categorical variables.
Clinical data correspond to information available at the inclusion visit in the ASCOT-BPLA trial.
Biomarker data presented in the table are those available at V2.
Legend: SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL, low-density cholesterol; eGFR, estimated glomerular filtration rate; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; LVH, left ventricular hypertrophy based on information from investigator electrocardiogram; PIIINP, N-Terminal Propeptide of Type III Collagen; PICP, procollagen I carboxyterminal propeptide; CITP, carboxyl-terminal telopeptide of collagen type I; MMP1, matrix-metalloproteinase 1; NT-pro BNP, N-terminal pro brain natriuretic peptide; hsTnT, high-sensitivity troponin T.

Table 2. Matched- and within-person biomarker adjusted changes

			Adjusted mean of the absolute change and its 95%CI	
		Matched between-person analysis*		
Studied biomarker	beta estimate (95%CI)	p	Control group	Spironolactone group
PIIINP, ng/mL	-0.93 [-1.77 ; -0.09]	0.031	0.52 [-0.05 ; 1.09]	-0.41 [-0.97 ; 0.16]
PICP, ng/mL	-10.9 [-20.3 ; -1.50]	0.023	4.54 [-1.77 ; 10.9]	-6.36 [-12.5 ; -0.21]
CITP, ng/mL	1.16 [-0.14 ; 2.45]	0.080	-1.19 [-2.06 ; -0.32]	-0.03 [-0.88 ; 0.81]
PIIINP/CITP ratio	-0.39 [-0.75 ; -0.03]	0.034	0.38 [0.14 ; 0.63]	-0.01 [-0.24 ; 0.22]
MMP1, ng/mL	-0.10 [-0.54 ; 0.35]	0.66	0.23 [-0.06 ; 0.53]	0.14 [-0.16 ; 0.43]
CITP/MMP1 ratio	0.09 [-0.55 ; 0.73]	0.78	-0.38 [-0.82 ; 0.06]	-0.29 [-0.70 ; 0.13]
NTproBNP, pg/mL	11.9 [-74.4 ; 98.3]	0.78	26.3 [-32.6 ; 85.2]	38.2 [-19.2 ; 95.6]
hsTnT, pg/mL	0.41 [-1.40 ; 2.22]	0.65	0.56 [-0.68 ; 1.80]	0.97 [-0.23 ; 2.17]
		Within-person analysis**		
Studied biomarker	beta estimate (95%CI)	p	Control period	Spironolactone period
PIIINP, ng/mL	-0.27 [-0.78 ; 0.25]	0.31	0.08 [-0.25 ; 0.40]	-0.19 [-0.50 ; 0.12]
PICP, ng/mL	-11.8 [-17.5 ; -6.1]	< 0.0001	3.63 [0.08 ; 7.18]	-8.2 [-11.7 ; -4.7]
CITP, ng/mL	0.20 [-0.65 ; 1.06]	0.64	-0.45 [-0.97 ; 0.08]	-0.24 [-0.76 ; 0.28]
PIIINP/CITP ratio	-0.36 [-1.20 ; 0.49]	0.41	0.28 [-0.27 ; 0.82]	-0.08 [-0.61 ; 0.45]
MMP1, ng/mL	-0.06 [-0.55 ; 0.42]	0.79	-0.05 [-0.37 ; 0.26]	-0.12 [-0.43 ; 0.19]
CITP/MMP1 ratio	-0.01 [-0.67 ; 0.66]	0.99	-0.22 [-0.62 ; 0.17]	-0.23 [-0.62 ; 0.16]
NTproBNP, pg/mL	-53.8 [-82.1 ; -25.4]	0.0003	32.8 [15.5 ; 50.1]	-20.9 [-39.0 ; -2.86]
hsTnT, pg/mL	-0.36 [-1.44 ; 0.72]	0.51	0.80 [0.18 ; 1.43]	0.44 [-0.18 ; 1.07]

*Models adjusted on V2 biomarker levels, age, gender and propensity score.

**Models adjusted on V1 biomarker levels for control period and V2 biomarker levels for spironolactone period.

Legend: PIIINP, N-Terminal Propeptide of Type III Collagen; PICP, procollagen I carboxyterminal propeptide; CITP, carboxyl-terminal telopeptide of collagen type I; MMP1, matrix-metalloproteinase 1; NT-pro BNP, N-terminal pro brain natriuretic peptide; hsTnT, high-sensitivity troponin T.