**The Effect of Spironolactone on**

**Cardiovascular Function and Markers of Fibrosis in**

**People at Increased Risk of Developing Heart Failure:**

**The Heart “OMics” in AGEing (HOMAGE) Randomised Clinical Trial**

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**Key-Points**

**(100 words)**

**Question:** Does spironolactone alter collagen turnover in people at increased risk of heart failure?

**Findings:** 527 patients with or at high-risk of coronary disease and a plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) >125ng/L were randomised to spironolactone or control. Spironolactone had favourable effects on blood pressure, NT-proBNP, left atrial volume and type-I collagen metabolism, but not on the primary endpoint, serum procollagen type-III N-terminal pro-peptide. Effects were similar when serum galectin-3, a possible marker of fibrotic activity, was above or below median.

**Meaning:** Whether the ability of spironolactone to inhibit myocardial fibrosis delays progression to heart failure requires further investigation.

**Abstract (342 words)**

**Importance:** Cardiovascular accumulation of collagen (fibrosis) may contribute to the progression from ventricular dysfunction to heart failure. Galectin-3, a potential marker of pro-fibrotic activity, might identify those at greater risk.

**Objective:** To investigate the effects of spironolactone, according to serum galectin-3 concentration, on serum markers of fibrosis and on cardiac structure and function, in people at increased risk of developing heart failure.

**Design:** Prospective, randomized, open-label, blinded endpoint (PROBE) trial.

**Setting:** Clinical research facilities in ten European hospitals.

**Participants:** People with, or at high-risk of, coronary disease with increased plasma concentrations of B-type natriuretic peptides (BNP or NT-proBNP).

**Interventions:** spironolactone (up to 50 mg/day) or control for up to nine months.

**Main Outcomes and Measures:** The primary outcome was the interaction between baseline serum galectin-3 and change in serum procollagen type-III N-terminal pro-peptide (PIIINP), a by-product of type-III collagen synthesis. Serum procollagen type-I C-terminal pro-peptide (PICP) and collagen type-1 C-terminal telopeptide (CITP), respectively reflecting synthesis and degradation of type-I collagen, were also measured.

**Results:** Of 527 participants, the median age was 73 years and 26% were women. Median follow-up was 267 days. Changes in PIIINP were similar for those assigned to spironolactone and control (mean difference -0.15; 95% confidence interval [CI] -0.44 to 0.15 μg/L; p=0.32) and did not differ when serum galectin-3 was above or below median. Those assigned to spironolactone had greater declines in PICP (mean difference -8.1; -95% CI -11.9 to -4.3 μg/L; p<0.0001) and PICP/CITP ratio (mean difference -2.9; 95% CI -4.3 to -1.5; <0.0001). Systolic blood pressure (mean difference -10; 95% CI -13 to -7 mmHg; p<0.0001), left atrial volume (mean difference -1; 95% CI -2 to 0 mL/m2; p=0.010) and NT-proBNP (mean difference -57; 95% CI -81 to -33 ng/L; p<0.0001) were lower on spironolactone at the final assessment.

**Conclusions and Relevance:** Spironolactone reduced PICP/CITP ratio, consistent with reduced synthesis and increased degradation of type-I collagen, and reduced NT-proBNP and left atrial volume, suggesting favourable effects on cardiac function. Further research is required to determine whether spironolactone can delay or prevent progression to symptomatic heart failure.

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Two tables

Three figures (including CONSORT diagram)

**Introduction**

Many people with cardiovascular disease will develop heart failure, leading to substantial disability, demands on health-services and mortality (1) (2). Early identification of cardiac dysfunction and therapeutic targeting of specific pathways of disease progression, such as myocardial and vascular fibrosis (3-5) (6-12), might delay or prevent the onset of heart failure.

Mineralo-corticoid receptor antagonists (MRA) improve cardiac structure, function and prognosis in patients with a reduced left ventricular ejection fraction (LVEF) and heart failure (HFrEF), and perhaps also when LVEF is preserved (HFpEF) (13-15). MRA also reduce serum markers of collagen synthesis in patients with a range of cardiovascular diseases, including HFrEF and HFpEF (9, 11), which might reflect favourable effects on fibrosis. Galectin-3 is a proposed marker of fibrotic activity (16, 17) and, in experimental models, mediator of aldosterone-induced fibrosis (18). In the general population, higher plasma concentrations of galectin-3 predict the development of heart failure and, subsequently, a worse outcome (19, 20).

Accordingly, we investigated whether spironolactone had favourable effects on serum markers of collagen metabolism in people at increased risk of developing heart failure and whether the effect was greater in patients with higher serum concentrations of galectin-3.

**Methods**

**Trial Design and Oversight**

Heart “OMics” in AGEing (HOMAGE) is a research consortium, based in Europe, investigating biomarkers for predicting incident heart failure in older people and bio-targets for preventing it; assets include several large clinical data-bases and collections of biological material (21, 22). The HOMAGE clinical trial was a prospective, randomised, open-label, blinded-endpoint (PROBE) multi-centre trial, investigating the effects of MRA on markers of collagen metabolism and cardiovascular structure and function in people at increased risk of developing heart failure (11). The protocol and statistical analysis plan are available at <https://clinicaltrials.gov/ct2/show/NCT02556450>. The trial was funded by the European Union 7th Framework Programme for Research and Technological Development (grant: 305507 <http://www.homage-hf.eu>). The sponsor was ACS Biomarkers (Amsterdam, The Netherlands). The European Drug Development Hub (EDDH) (Nancy, France) managed monitoring and data-collection. The trial was approved by relevant ethics committees and regulatory bodies. Participants provided written, informed consent. An executive committee developed the protocol, oversaw trial conduct, and interpreted the results. A clinical endpoints committee adjudicated hospitalisations and deaths blind to assigned treatment. An independent data monitoring committee oversaw safety.

**Trial Population**

People of either sex, aged ≥65 years (amended to ≥60 years) at increased risk of developing heart failure because they had, or were at high risk of, coronary disease were screened. Those with a plasma amino-terminal pro-B-type natriuretic peptide (NT-proBNP) of 125-1,000 ng/L or BNP 35-280 ng/L were eligible for randomisation provided none of the exclusion criteria were met. This ‘window’ excluded people at low risk of developing heart failure and those with advanced disease requiring further investigation and treatment. The main exclusion criteria ***(Supplementary Table 1)*** were an estimated glomerular filtration rate (eGFR) <30 mL/minute/1.73m2 (23), serum potassium >5.0 mmol/L, LVEF <45%, atrial fibrillation, a diagnosis of heart failure or treatment with loop diuretics. Background therapy could include any conventional treatment except loop diuretics, MRA or other potassium-sparing diuretics.Other treatments for concomitant conditions, such as hypertension, diabetes mellitus or coronary disease, were permitted.

**Aims and Endpoints**

Serum concentration of procollagen type-III N-terminal pro-peptide (PIIINP), thought to reflect type-III collagen synthesis, was chosen as the marker of response, based on a landmark trial of spironolactone for HFrEF (9). Serum galectin-3 was chosen as a marker of fibrotic activity (16, 17). The primary endpoint was the interaction between changes in PIIINP from baseline to final visit and baseline galectin-3.

Secondary aims were to investigate the effects of spironolactone on other serum markers of collagen metabolism, on cardiac structure and function (assessed by echocardiography and NT-proBNP), and on exercise capacity. Specific secondary endpoints included changes in serum markers of type-I collagen synthesis (procollagen type-I C-terminal pro-peptide; PICP) and degradation (collagen type-1 C-terminal telopeptide; CITP), galectin-3 and NT-proBNP; echocardiographic left atrial volume and left ventricular mass; Doppler measures of cardiac function; tricuspid annular plane systolic excursion (TAPSE) and an incremental shuttle walk-test (24). Safety endpoints included incidences of serum potassium >5.5 or <3.5 mmol/L, decline in eGFR by >20%, and a clinical composite of heart failure or atrial fibrillation, non-fatal myocardial infarction or stroke or cardiovascular death (25).

**Laboratory Assays**

Blind to clinical data and randomization, PIIINP and CITP were measured by radio-immunoassay (Orion Diagnostica®), PICP by enzyme immune-assay (METRA; Quidel Corporation®) and matrix metalloproteinase-1 (MMP-1) by an amplified luminescent proximity homogenous assay (ALPHA-LISA) (PerkinElmer®).. Galectin-3 was measured by enzyme-linked immunosorbent assay (ELISA) (BG Medicine®) and NT-proBNP, high-sensitivity troponin T (hsTnT) and growth differentiation factor 15 (GDF-15) by electro-chemi-luminescence (ELECSYS® 2010 analyser; Roche Diagnostics, Mannheim, Germany). All intra-assay variations were less than 10%.

**Echocardiography**

Echocardiograms were recorded, de-identified and transferred to a core laboratory (University Hospital of Nancy). Blind to treatment allocation, a single experienced echocardiographer (Erwan Bozec) measured variables using dedicated software (Echo PAC, GE Healthcare). Measurements were repeated at least two months later, blind to the first measurement. All recordings with suboptimal images and/or with differences >10% were reviewed by a senior cardiologist (Nicolas Girerd) to exclude measurement error.

**Randomisation and Blinding**

Participants were randomised by a coordinating centre (Leuven) using statistical software (SAS 9.4), web-based management system and random, permuted blocks, stratified by site. Spironolactone was initiated at 25 mg/day. Doses could be increased up to 50 mg/day or reduced to 25 mg every other day or stopped with or without re-initiation according to serum potassium and renal function ***(Supplementary Table 2).*** Those assigned to the control group received no additional treatment. All core-laboratory staff and the clinical endpoints committee were blind to treatment allocation, but investigators were not.

**Follow-up**

After randomisation, follow-up visits were planned after one week and at one, two, three, six and nine months, to assess serum potassium, renal function and blood pressure. At the one-month and final visits, baseline assessments were repeated, although echocardiography was not mandated at one month. The final visit was planned to occur at nine months. Due to slow recruitment, enrolment was extended but due to the fixed funding-duration, the final visit occurred between three and eight months for some participants***.***

**Statistical Analysis**

Sample-size was based on a test for interaction by analysis of variance (26) and required 800 participants to detect an interaction term of 0.79 μg/L between PIIINP and median galectin-3 with a two-sided significance of 5% and 90% power, given a residual standard deviation for PIIINP of 1.73 μg/L (27). Analyses (Stata® version 15.1) used the intention-to-treat principle. Baseline characteristics were summarised for categorical variables using frequencies and percentages, and for continuous variables using median and interquartile range (IQR, defined as the 1st and 3rd quartiles). Analysis of covariance (ANCOVA) was used for the primary endpoint. A linear regression model was fitted, including variables to indicate treatment group, galectin-3 above or below median, and baseline PIIINP. An interaction term was included to evaluate the effect of spironolactone when galectin-3 was above median. Residual analysis was used to examine the fit of the model to the assumptions of linear regression with data transformed as required. Secondary endpoints were analysed using ANCOVA for continuous data or multi-variable logistic regression for composite clinical events. No adjustments were made for multiple comparisons to allow for type-1 error in view of the exploratory nature of this proof-of-concept trial.

**Results**

Between January 2016 and June 2018, 877 patients were consented, of whom 561 were eligible and 527 were randomised ***(Figure 1 – Consort Diagram; Supplementary Table 3).*** The main reason for exclusion was NT-proBNP <125ng/L or BNP <35ng/L. Baseline-characteristics of those assigned to spironolactone or control were similar ***(Table 1).*** Median age was 73 (IQR 68 to 78) years, 26% were women, 72% had coronary disease, 42% had diabetes and 22% had an eGFR <60 mL/minute/1.73m2. Most participants (78%) had a history of hypertension, were overweight or obese and, despite receiving two or more anti-hypertensive medications, 50% had a systolic blood pressure >140mmHg. Participants reported breathlessness at moderate levels of exertion and the shuttle walk-test ***(Table 1)*** was mildly impaired (50; IQR 32-70 completed shuttles) compared to published normal values (mean ± SD for general population age ≥70 years is 63±19 shuttles) (24). The rise in heart rate with exercise was modest (many participants were receiving beta-blockers) but systolic blood pressure rose from 140 (IQR 127 to 155) mmHg to 165 (IQR 144 to 187) mmHg. Left ventricular volumes, ejection fraction and mass were normal but left atrial volume (31 (IQR 26–37) mL/m2), plasma NT-proBNP (median 214; IQR 137–356 ng/L) (28) and serum galectin-3 (median 16.1; IQR 13.5–19.7 µg/L) were increased (19). Serum concentrations of markers of collagen metabolism were not greater than published normal values but this could reflect differences amongst assays (11, 29). Participants with a serum galectin-3 above median had higher serum CITP and NT-proBNP but similar PIIINP and PICP.

**Follow-Up**

Data were acquired on 506 (96%) at the final visit; 345 (68%) had >250 days of follow-up ***(Supplementary Figure 1)***. At the one-month visit, of those assigned to spironolactone, 99 were prescribed 50mg/day, 118 were prescribed 25mg/day, 26 were prescribed 25mg every other day, ten were not prescribed spironolactone and information on dose was missing for twelve. No participant assigned to the control group was prescribed an MRA. During the trial, serum potassium exceeded 5.5mmol/L in seven participants assigned to spironolactone but only one developed a value >6.0 mmol/L. Two participants in each group died. Fourteen clinical composite endpoints occurred in eleven participants assigned to placebo and twelve endpoints in nine participants assigned to spironolactone (p=0.50) ***(Supplementary Table 4).***

**Primary End Point and Other Markers of Fibrosis**

Serum PIIINP changed little during follow-up, with a mean difference between groups from baseline to the final visit of -0.15 μg/L (95% CI -0.44 to 0.15; p = 0.323); there was no interaction (p = 0.947) with galectin-3 ***(Figure 2; Table 2)***. However, in those assigned to spironolactone, there was a greater decline in serum PICP (mean difference -8.1 μg/L; 95% CI -11.9 to -4.3; p <0.001) and increase in CITP and consequently, a greater decline in PICP/CITP ratio (mean difference -2.9; 95% CI -4.3 to -1.5; p <0.001) but, again, no interaction with galectin-3 was observed ***(Figure 2; Table 2).*** Serum MMP-1 did not change. Serum galectin-3 increased at both time-points for those assigned to spironolactone ***(Table 2).***

**Secondary End Points**

Systolic blood pressure (mean difference -10 mmHg; 95% CI -13 to -7; p <0.001) and plasma NT-proBNP (mean difference -41 ng/L; 95% CI -75 to -11; p=0.009) were lower at the final visit in those assigned to spironolactone but symptoms, exercise capacity, haemoglobin, troponin and GDF-15 were no different ***(Figure 3; Table 2)***. Serum concentrations of sodium fell, and serum potassium, urea and creatinine rose on spironolactone. Small reductions in QRS duration (p=0.003), left atrial volume index (p=0.010), left ventricular mass index (p=0.079) and early mitral flow (E-wave) velocity (p <0.001) and increases in LVEF (p=0.022) were observed in those assigned to spironolactone ***(Table 2)*** but the ratio of E to early diastolic tissue velocity (e’) did not change. At one month, results were generally similar to those observed at the end of the trial.

**Discussion**

In contrast to some previous reports, we did not show reductions in serum PIIINP after administration of spironolactone nor did we observe an interaction with baseline serum galectin-3 (11). However, we did observe a decline in serum PICP and a rise in CITP with spironolactone, suggesting, respectively, reduced synthesis and increased degradation of type-I collagen (10, 30-32). Changes in PICP and CITP were prominent within one month, persisted and were accompanied by reductions in left atrial volume and increases in LVEF in the longer term, indicating favourable effects on cardiac structure and function.

Type-I collagen comprises large-diameter fibres with a high propensity for cross-linking that make a substantial contribution to myocardial stiffness compared to the finer type-III collagen fibres (10). Pathological myocardial fibrosis is characterised by an excess of type-I compared to type-III collagen that, along with hypertrophy, contributes to a restrictive ventricular pathophysiology, leading to increases in diastolic ventricular pressures and atrial dilation that may culminate in HFpEF (10, 11, 31, 33). Our data suggest that MRA might reduce or reverse accumulation of type-I collagen but have little or no effect on type-III collagen, effects that might be considered advantageous for an intervention aimed at preventing or reversing pathological myocardial fibrosis.

We are aware of only one study that obtained myocardial biopsies before and after administration of spironolactone in patients with heart failure (34), where a serum PICP/CITP of >35, rare amongst participants in our trial, had more myocardial fibrosis and a greater abundance of both type-I and type-III collagen. Spironolactone reduced both PICP/CITP ratio and myocardial collagen fraction. Several other trials have investigated the effects of spironolactone on serum collagen markers in a broad range of cardiovascular diseases (11), generally showing that serum PIIINP was not markedly different in people with and without cardiovascular disease but that administration of MRA to patients with severe HFrEF reduced serum PIIINP, which why it was chosen as the primary efficacy marker for HOMAGE (11). However, in one substantial trial of patients with less severe HFrEF, canrenone, the active metabolite of spironolactone, did not reduce serum PIIINP, despite reducing left ventricular mass, left atrial diameter and B-type natriuretic peptide and increasing LVEF (35). Differences between assays or disease-state may account for these apparent inconsistencies.

Serum PICP is raised in people with cardiovascular disease and also declines with administration of an MRA (11, 36). Fewer trials have investigated the effects of MRA on serum CITP and found no consistent effect (11). Duration of treatment may be important; we observed clearer reductions in CITP with spironolactone at one month compared to the final visit, which might reflect an early increase in the rate of collagen turnover before it subsides to a new steady-state. We observed a rise in galectin-3 with the administration of spironolactone as have others (37). This might reflect increased galectin-3 production due to MR blockade-mediated increases in aldosterone subsequent to administration of an MRA (38), or reduced galectin-3 clearance due to the decline in eGFR.

Spironolactone reduced collagen marker within a few weeks, suggesting that they reflect changes in the rate of turnover rather than the mass of collagen. However, a favourable effect on turnover should eventually reduce fibrosis. Blood concentrations of biomarkers reflect the equilibrium between production and disposal. Plasma concentrations of biomarkers cleared by the kidney should rise as eGFR declines with the introduction of MRA, suggesting that the decline in PICP is due to reduced production, which could reflect favourable effects of MRA on ventricular pre- and after-load or a direct effect of spironolactone on collagen production or processing (30)(39). Many of our participants had inadequately controlled blood pressure, which fell substantially in those assigned to spironolactone, as observed in trials of resistant hypertension (40), and was accompanied by a decline in NT-proBNP and, in the longer term, echocardiographic evidence of cardiac remodelling. However, the heart contains only a small proportion of the body’s collagen. If spironolactone has a specific effect only on cardiovascular collagen, then the effect would have to be very large in order to change serum marker concentrations. It is more likely that changes in serum PICP indicate an effect on fibroblasts in many organs (10).

Assuming an extra-cellular fluid volume of 15 litres, a rise in serum potassium concentration of just 0.2mmol/L, as we observed, would require retention of only 3-4 mmol of potassium. If MRA cause retention of much larger amounts, as is likely (41), potassium must be transferred to the intra-cellular space and, therefore, assuming no change in intra-cellular potassium concentration, intra-cellular water must increase (42). Accordingly, changes in weight may underestimate the effect of spironolactone on extra-cellular water. NT-proBNP and left atrial volume both declined on spironolactone, implying a reduction in cardiac filling pressures that might be due to a subtle reduction in plasma volume. Although haemoglobin did not change, serum sodium concentration fell, implying even greater loss of extra-cellular sodium than of water.

Our population had normal left ventricular end-diastolic volumes and LVEF but left atrial volume and left ventricular mass were increased. We excluded patients with a diagnosis of heart failure or atrial fibrillation and those taking loop diuretics but, with the exception of E/e’, our patients had more severe cardiac dysfunction than many of those enrolled in trials of HFpEF (11). Many of our patients also reported breathlessness on moderate exertion and had reduced exercise capacity and therefore fulfilled all of the diagnostic criteria for HFpEF; symptoms, reduced exercise capacity, cardiac dysfunction and raised NT-proBNP. Spironolactone reduced left atrial volume and NT-proBNP, suggesting favourable effects on cardiac structure and function that could delay or prevent the onset of clinically overt heart failure, although our trial was neither large nor long enough to demonstrate such an effect and no differences in symptoms or exercise capacity were observed.

The HOMAGE trial did not enrol the pre-specified number of participants or follow all those included for the intended duration. However, even if we had conducted the trial as originally planned a different result is unlikely. Our trial was a mechanistic proof-of-concept trial and should be interpreted in the context of information from other trials.

In conclusion, spironolactone did not reduce serum PIIINP and no interaction was observed with galectin-3. The effect of spironolactone on PICP/CITP ratio appears early and is sustained, potentially make PICP/CITP a useful biomarker for future research on the effects of interventions on collagen metabolism. The effects of spironolactone on type-I collagen turnover, blood pressure and cardiac function in people with a cardiovascular profile consistent with clinically occult HFpEF, indicates the need for clinical trials to determine whether MRA can delay or prevent progression to clinically overt heart failure.

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**Legends to Figures**

**Figure 1**

Changes from baseline to one month and final visits for serum concentrations of

Panel A: procollagen type-III N-terminal pro-peptide (PIIINP)

Panel B: PIIINP for those with a baseline serum galectin above or below median.

Panel C: procollagen type-I C-terminal pro-peptide (PICP)

Panel D: collagen type-1 C-terminal telopeptide (CITP)

Panel E: the ratio of PICP to CITP

Panel F: Galectin-3

Data shown are mean change and standard deviation.

**Figure 2**

Changes from baseline to one month and to the final visit in:-

Panel A: Systolic blood pressure.

Panel B: Plasma concentrations of NT-proBNP

Panel C: Left atrial volume index

Panel D: E/A ratio

Panel E: Serum potassium

Panel F: estimated glomerular filtration rate

Data shown are mean change and standard deviation.

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