ESC European Society of Cardiology

European Journal of Heart Failure (2022) **24**, 321–331
doi:10.1002/ejhf.2394

RESEARCH ARTICLE

Biomarker-based assessment of collagen cross-linking identifies patients at risk of heart failure more likely to benefit from spironolactone effects on left atrial remodelling. Insights from the HOMAGE clinical trial

Susana Ravassa[1,2†], Begoña López[1,2†], João Pedro Ferreira[3], Nicolas Girerd[3], Erwan Bozec[3], Pierpaolo Pellicori[4], Beatrice Mariottoni[5], Franco Cosmi[5], Mark Hazebroek[6], Job A.J. Verdonschot[6], Joe Cuthbert[7], Johannes Petutschnigg[8], María U. Moreno[1,2], Stephane Heymans[6], Jan A. Staessen[9,10], Burkert Pieske[8,11], Frank Edelmann[8], Andrew L. Clark[7], John G.F. Cleland[4], Faiez Zannad[3], Javier Díez[1,2,12*], and Arantxa González[1,2*], on behalf of the HOMAGE Trial Committees and Investigators

[1]Program of Cardiovascular Diseases, CIMA, Universidad de Navarra and IdiSNA, Pamplona, Spain; [2]CIBERCV, Carlos III Institute of Health, Madrid, Spain; [3]Université de Lorraine, Inserm, Centre d'Investigation Clinique Plurithématique 1433, U1116, CHRU de Nancy, F-CRIN INI-CRCT, Nancy, France; [4]Robertson Centre for Biostatistics, Institute of Health and Wellbeing, University of Glasgow, Glasgow Royal Infirmary, Glasgow, UK; [5]Department of Cardiology, Cortona Hospital, Arezzo, Italy; [6]Department of Cardiology, Maastricht University Medical Center, Maastricht, The Netherlands; [7]Department of Cardiology, University of Hull, Castle Hill Hospital, Cottingham, UK; [8]Department of Internal Medicine and Cardiology Campus Virchow Klinikum, Charité University Medicine Berlin and German Centre for Cardiovascular Research (DZHK), Partner Site Berlin, Berlin, Germany; [9]Non-Profit Research Institute Alliance for the Promotion of Preventive Medicine, Mechelen (APPREMED), Mechelen, Belgium; [10]Biomedical Sciences Group, Faculty of Medicine, University of Leuven, Leuven, Belgium; [11]Berlin Institute of Health, Berlin, Germany; and [12]Departments of Nephrology and Cardiology, Clínica Universidad de Navarra, Pamplona, Spain

Received 27 May 2021; revised 19 October 2021; accepted 22 November 2021; online publish-ahead-of-print 9 December 2021

Aims

The HOMAGE randomized trial found that spironolactone reduced left atrial volume index (LAVI), E:A ratio, and a marker of collagen type I synthesis (procollagen type I C-terminal propeptide) in patients at risk of heart failure (HF). Previous trials showed that patients with HF, preserved ejection fraction and low serum collagen type I C-terminal telopeptide to matrix metalloproteinase-1 ratio (CITP:MMP-1), associated with high collagen cross-linking, had less improvement in diastolic function with spironolactone. We evaluated the interaction between serum CITP:MMP-1 and spironolactone on cardiac function in the HOMAGE trial.

Methods and results

Patients at risk of HF were randomized to spironolactone (n = 260) or not (n = 255). Blood sampling and echocardiography were done at baseline, one and nine months. CITP:MMP-1 was used as an indirect measure of collagen cross-linking. Higher baseline CITP:MMP-1 (i.e. lower collagen cross-linking) was associated with greater reductions in LAVI with spironolactone at both one (p = 0.003) and nine (p = 0.01) months, but no interaction was observed for E:A ratio. Spironolactone reduced LAVI after one and nine months only for those patients in the third

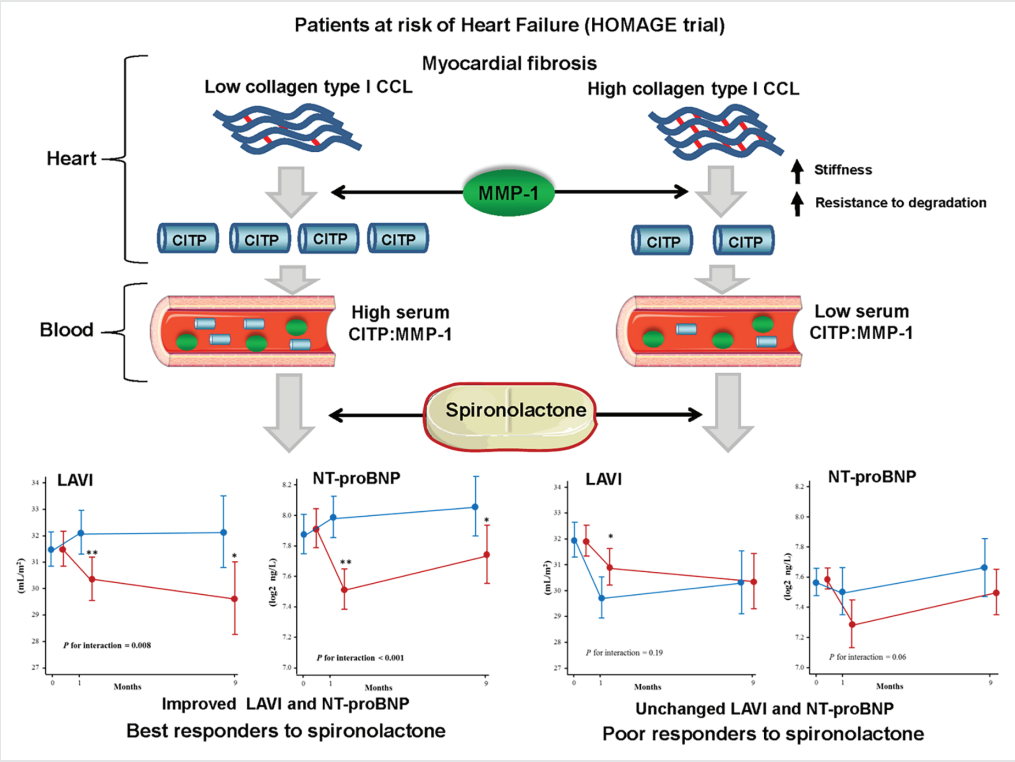
*Corresponding authors. Program of Cardiovascular Diseases, CIMA. Universidad de Navarra, Avda Pio XII 55, Pamplona 31008, Spain. Email: amiqueo@unav.es or jadimar@unav.es

†Both authors contributed equally to this work.

tertile of CITP:MMP-1 (estimated lowest collagen cross-linking) [mean differences$_{spiro/control}$: −1.77 (95% confidence interval, CI −2.94 to −0.59) and −2.52 (95% CI −4.46 to −0.58) mL/m^2; interaction $p_{across\text{-}tertiles}$ = 0.005; interaction $p_{third\ tertile}$ = 0.008] with a similar trend for N-terminal pro-B-type natriuretic peptide which was consistently reduced by spironolactone only in the lowest collagen cross-linking tertile [mean differences$_{spiro/control}$: −0.47 (95% CI −0.66 to −0.28) and −0.31 (95% CI −0.59 to −0.04) ng/L; interaction $p_{across\text{-}tertiles}$ = 0.09; interaction $p_{third\ tertile}$ < 0.001].

Conclusions These findings suggest that, for patients at risk of HF, the effects of spironolactone on left atrial remodelling may be more prominent in patients with less collagen cross-linking (indirectly assessed by serum CITP:MMP-1).

Graphical Abstract



Patients at risk of heart failure from the HOMAGE clinical trial were classified according to the baseline degree of myocardial collagen cross-linking, non-invasively assessed by the serum collagen type I C-terminal telopeptide (CITP) to matrix metalloproteinase-1 (MMP-1) ratio (CITP:MMP-1). As highly cross-linked collagen fibres are more resistant to degradation and CITP is a cross-linked peptide, for a given MMP-1 quantity less CITP will be released and, subsequently, serum CITP:MMP-1 will be lower. Whereas patients with low collagen cross-linking (high CITP:MMP-1) benefit from the cardioprotective effects of treatment with spironolactone on left atrial remodelling [i.e. a decrease in left atrial volume index (LAVI)] and on N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels, these beneficial effects are not found in patients with higher collagen cross-linking (low CITP:MMP-1).

Keywords Heart failure • Spironolactone • Atrial remodelling • Collagen cross-linking

Introduction

Prevention of heart failure (HF) before symptoms develop may be better than waiting for symptoms of advanced disease, when the prognosis could be more difficult to modify.[1] In this context, the multicentre, randomized, Heart 'OMics' in AGEing (HOMAGE) trial recently showed, for patients at risk of HF, that spironolactone reduced left atrial volume index (LAVI), early mitral flow velocity

(E wave) and consequently the E:A ratio.[2] Additionally, spironolactone reduced plasma concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP), and serum procollagen type I C-terminal propeptide (PICP), a marker of collagen type I synthesis and, potentially, of myocardial fibrosis.[2]

Left atrial (LA) remodelling, as evidenced by an increase in LAVI, has been proposed as a biomarker of the duration and severity of left ventricular (LV) diastolic dysfunction, reflecting the cumulative effect of prolonged increases in filling pressure.[3] In addition, LA enlargement is associated with an increased risk of atrial fibrillation (AF) and, for patients with HF, a worse prognosis.[4,5]

Myocardial interstitial fibrosis is a major pathophysiological mechanism involved in HF, increasing myocardial stiffness and contributing to LA remodelling[6] and LV diastolic dysfunction.[6,7] Myocardial fibrosis occurs when collagen deposition exceeds degradation. The functional impact of myocardial fibrosis depends not only on the quantity of collagen fibres deposited (mainly type I fibres) but also on their degree of cross-linking.[8] Increased myocardial collagen cross-linking (CCL) increases the stiffness and resistance to degradation of collagen fibres,[9] and has been associated with increased LV filling pressures and diastolic dysfunction in patients with HF and preserved ejection fraction (HFpEF).[7] We have identified a serum biomarker of CCL, the collagen type I C-terminal telopeptide to matrix metalloproteinase-1 ratio (CITP:MMP-1), which we have found to be inversely related to tissue-assessed myocardial CCL, and might be considered as an indirect biomarker of the degree of CCL.[10] Increased CCL makes collagen fibres more resistant to proteolytic degradation,[11,12] hindering degradation by MMP-1. Therefore, for a given quantity of MMP-1, more CCL will limit degradation, with a diminished release of collagen type I-derived peptide CITP.

For patients with HFpEF, the aldosterone receptor blockade in a diastolic HF (Aldo-DHF) trial showed that the effects of spironolactone on echocardiographic measurements of diastolic function (i.e. E:e′ ratio) were restricted to patients with a high serum CITP:MMP-1 (i.e. low CCL).[13] We sought to confirm and extend these findings for patients at risk of HF enrolled in the HOMAGE randomized trial.[2]

Methods

Trial design and population

The HOMAGE trial had a prospective, randomized, open-label, blinded endpoint (PROBE), multicentre design, in which patients at increased risk of developing HF were randomly assigned to receive spironolactone or not in addition to standard care (ClinicalTrials.gov Identifier: NCT02556450).[2,14] The investigation conformed with the principles outlined in the Declaration of Helsinki and the study was approved by all relevant ethics committees and regulatory bodies. All participants provided written informed consent prior to study-specific procedures. The main inclusion criteria were age 60 years or older, increased risk of cardiac dysfunction (defined by the presence of coronary artery disease or at least two of the following: diabetes, treated hypertension, microalbuminuria, abnormal electrocardiogram), and evidence of cardiac dysfunction (defined as an NT-proBNP between 125 and 1000 ng/L or BNP between 35 and 280 ng/L). The main exclusion criteria were estimated glomerular filtration rate (eGFR) <30 ml/min/1.73 m^2, serum potassium >5.0 mmol/L, LV ejection fraction <45%, a previous diagnosis of HF or treatment with loop diuretics and atrial fibrillation/flutter prior to randomization. Patients with an NT-proBNP >1000 ng/L were considered in need of investigation for serious underlying cardiac or renal disease rather than inclusion in the trial.

Of 527 patients included in HOMAGE, 515 had CITP:MMP-1 measurements available and were included in this analysis (260 randomized to spironolactone and 255 to standard of care). No differences in clinical and echocardiographic parameters were found between patients with or without biomarker measurements (data not shown). The median (percentile$_{25-75}$) follow-up was 8.9 (6.0–9.2) months. At baseline, at 1 month and at the end of the study, participants had clinical, biomarker and echocardiographic measurements recorded.[14]

Echocardiography

Echocardiograms were recorded, de-identified and transferred to a core laboratory (University Hospital of Nancy). Blind to treatment allocation, a single experienced echocardiographer measured variables using dedicated software (Echo PAC, GE Healthcare). Measurements were repeated at least 2 months later, blind to the first measurement. All recordings with suboptimal images and/or with differences >10% were reviewed by a senior cardiologist (N.G.) to exclude measurement error.[2]

Biomarkers

Commercial radio-immunoassays (Orion Diagnostica) were used to measure CITP and procollagen type III N-terminal propeptide (PIIINP). The lower limit of detection was 0.6 ng/mL for CITP and 0.3 ng/mL for PIIINP. Serum MMP-1 was measured by an AlphaLISA (PerkinElmer) with a lower limit of detection of 0.08 ng/mL. Serum PICP was measured using the METRA EIA kit (Quidel Corporation) with a lower limit of detection of 0.2 ng/mL. All inter- and intra-assay coefficients of variation were <10%. The CITP:MMP-1 ratio, an accepted measure of CCL, was calculated by dividing CITP by MMP-1 concentration in molar units.[10] In addition, plasma NT-proBNP and high sensitivity troponin T (hs-TnT) were measured by electro-chemiluminescent assays (Roche diagnostics).

Statistical analysis

Descriptive data are expressed as median with 25th and 75th percentiles, and categorical variables as numbers and percentages. Non-normally distributed variables were analysed after logarithmic transformation. Differences between randomized groups were tested by Student's *t*-test for unpaired data once normality was demonstrated. Otherwise, a non-parametric test (Mann–Whitney U test) was used. Categorical variables were analysed by chi-squared or Fisher's exact test. Linear tests for trend were used for subgroup analyses. Linear mixed models with a random intercept to account for intra-participant correlation of repeated measures were used to estimate changes over time. Treatment, visit, baseline CITP:MMP-1 (continuous or as tertiles), their interaction terms, and baseline values of the dependent variable were added as fixed effects to examine whether the degree of CCL modified the effects of spironolactone on variables of interest,

compared to controls. Because renal clearance of biomarkers may be important, in particular for CITP, analyses were also adjusted for eGFR. If the model performed better using 'visit' as a random slope, it was included in addition to the random intercept. The variance component structure was specified according to the best fit model, determined by the likelihood ratio test and Akaike information criterion. Between-group comparisons are presented as mean difference and 95% confidence intervals (CIs). Other variables associated with CITP:MMP-1 were used as covariates to adjust for the effects of spironolactone versus control. Residuals were examined to assess the assumptions of linear regression, transforming the data if required. The Benjamini and Hochberg multiple test correction (false discovery rate of 5%) was applied to the analyses testing the influence of baseline CITP:MMP-1 on the spironolactone effects on echocardiographic variables and biomarkers by tertiles of CITP:MMP-1. Mixed-effect analyses were performed with the 'mixed' command within STATA. Statistical significance was set as a 2-sided p of 0.05. Statistical analyses were done using SPSS (15.0 version) and STATA (13.0 version) software.

Results

Baseline characteristics

Patients were classified by tertiles of baseline CITP:MMP-1: first tertile: <0.932, second tertile: 0.932–1.820, third tertile: >1.820, corresponding to highest, medium and lowest CCL (*Table 1*). Most clinical and echocardiographic variables were similar across tertiles, but for patients in lower tertiles of CCL, eGFR was lower, and body mass index (BMI), tricuspid annular plane systolic excursion (TAPSE) and plasma or serum concentrations of hs-TnT, NT-proBNP, PIIINP and PICP were higher.

A total of 96 (56.1%), 76 (44.2%) and 88 (51.2%) patients were treated with spironolactone in the first, second and third CITP:MMP-1 tertiles, respectively ($\chi^2 = 4.95$, $p = 0.084$). There were no differences in demographic, clinical and biochemical variables between patients treated with spironolactone versus controls in any of the CITP:MMP-1 tertiles with the exception of a lower frequency of percutaneous coronary intervention, treatment with lipid-lowering drugs and a higher frequency of history of hypertension in those assigned to spironolactone compared to controls in the first tertile (online supplementary *Table S1*).

Influence of baseline CITP:MMP-1 ratio on spironolactone effects on left atrial volume index and E:A ratio in continuous interaction models

Continuous interaction analyses revealed that the reduction in LAVI in patients assigned to spironolactone was modified by baseline CITP:MMP-1 both after 1 month of treatment (interaction term = −1.17, 95% CI −1.94 to −0.41, $p = 0.003$) and at the end of the trial (interaction term = −1.32, 95% CI −2.32 to −0.31, $p = 0.010$), with LAVI decreasing more in patients with higher CITP:MMP-1 (potentially reflecting lower CCL). No significant modifying effect was found for the E:A ratio.

Effects of spironolactone on echocardiographic and clinical variables by CITP:MMP-1 tertiles

Analysis by tertiles also suggested that the effect of spironolactone on LAVI was modified by the degree of CCL at both 1 month and at the end of follow-up (interaction $p_{\text{accross-tertiles}} = 0.005$) (*Table 2*). In particular, in the first tertile (highest CCL), LAVI increased after 1 month of spironolactone as compared to controls (1.18, 95% CI 0.12 to 2.24 ml/m^2, $p = 0.029$), without further change by the end of trial (*Figure 1A*, *Table 2*). In the second tertile (medium CCL), LAVI was lower at 1 month (−1.62, 95% CI −2.70 to −0.53 ml/m^2, $p = 0.004$) for those assigned to spironolactone compared to controls, without further change by the end of trial (*Figure 1B*, *Table 2*). In the third tertile (lowest CCL), compared with controls, spironolactone reduced LAVI both at 1 month (−1.77, 95% CI −2.94 to −0.59 ml/m^2, $p = 0.003$) and at the end of trial (−2.52, 95% CI −4.46 to −0.58 ml/m^2, $p = 0.011$) (*Figure 1C*, *Table 2*). The influence of CCL on the effect of spironolactone on LAVI was independent of variables that were different across CITP:MMP-1 tertiles at baseline (BMI, coronary artery bypass graft, eGFR, TAPSE, hs-TnT, NT-proBNP, PICP, and PIIINP) (*Table 3*).

There was no significant interaction between CITP:MMP-1 tertiles and the effects of spironolactone on the E:A ratio. Nevertheless, compared with controls, spironolactone consistently reduced this variable only in patients in the third tertile (lowest CCL; *Table 2*).

Baseline CITP:MMP-1 tertiles did not affect the impact of spironolactone in any of the remaining echocardiographic (*Table 2*) and clinical variables (online supplementary *Table S2*). However, a trend for greater reduction in systolic blood pressure (interaction $p_{\text{accross-tertiles}} = 0.09$) was observed for patients in the second and third CITP:MMP-1 tertiles (online supplementary *Table S2*).

Effects of spironolactone on plasma/serum biomarkers by CITP:MMP-1 tertiles

There was a positive association between LAVI and NT-proBNP in the whole cohort with an increment of LAVI of 2.56 mL/m^2 (95% CI 1.77–3.36, $p < 0.001$) per doubling of NT-proBNP at baseline. A linear mixed effect model showed that doubling of NT-proBNP was associated with an increase in LAVI of 0.67 mL/m^2 (95% CI 0.43–0.90, $p < 0.001$) over time, independent of treatment and baseline LAVI. For patients in the highest tertile of CCL (first tertile), plasma NT-proBNP concentrations were not reduced by spironolactone, compared to controls, at either visit; for those in the middle tertile of CCL, spironolactone reduced NT-proBNP at 1 month but not at the trial end; for those in the third tertile (lowest CCL), spironolactone reduced NT-proBNP at both visits, compared to controls (interaction $p < 0.001$; *Figure 2*).

Spironolactone increased ($p < 0.05$) CITP:MMP-1 as compared with baseline values in the whole cohort, although this effect was not statistically significant compared with the change in the control group (online supplementary *Figure S1*). Changes in CITP:MMP1

Table 1 Demographic, clinical and biochemical characteristics at baseline in patients classified according to CITP:MMP1 tertiles

	CITP:MMP-1 tertiles			*p* for trend
	First (highest CCL) (*n* = 171)	Second (*n* = 172)	Third (lowest CCL) (*n* = 172)	
CITP:MMP-1 ratio	0.6 (0.4–0.8)	1.3 (1.1–1.5)	2.6 (2.1–4.1)	
Demographics				
Age, years	72 (67–77)	72 (69–77)	73 (68–79)	0.11
Women, *n* (%)	41 (24.0)	43 (25.0)	47 (27.3)	0.48
Current smoker, *n* (%)	13 (7.6)	13 (7.6)	15 (8.7)	0.74
Prior medical history, *n* (%)				
Hypertension	128 (74.9)	140 (81.4)	135 (78.5)	0.42
Diabetes mellitus	70 (40.9)	73 (42.4)	65 (37.8)	0.55
Coronary artery disease	126 (73.7)	119 (69.2)	125 (72.7)	0.84
Myocardial infarction	72 (42.1)	75 (43.6)	65 (37.8)	0.42
Percutaneous coronary intervention	96 (56.1)	85 (49.4)	80 (46.5)	0.08
Coronary artery bypass graft	38 (22.2)	43 (25.0)	54 (31.4)	0.054
Stroke/transient ischaemic attack	8 (4.7)	9 (5.2)	10 (5.8)	0.64
COPD	15 (8.8)	7 (4.1)	10 (5.8)	0.26
Baseline medications, *n* (%)				
ACE inhibitor	93 (54.4)	89 (51.7)	87 (50.6)	0.48
Angiotensin receptor blockers	42 (24.6)	51 (29.7)	50 (29.1)	0.35
Beta-blockers	111 (64.9)	121 (70.3)	127 (73.8)	0.07
Thiazide diuretics	28 (16.4)	39 (22.7)	20 (11.6)	0.24
Calcium channel blocker	33 (19.3)	43 (25.0)	32 (18.6)	0.87
Lipid-lowering drug	145 (84.8)	137 (79.7)	142 (82.6)	0.59
Aspirin	123 (71.9)	121 (70.3)	123 (71.5)	0.93
Any antiplatelet drug	133 (77.8)	133 (77.3)	137 (79.7)	0.67
Physical examination				
BMI, kg/m^2	27.8 (25.1–31.1)	28.2 (25.4–31.2)	28.6 (25.6–32.7)	**0.027**
HR, bpm	63.0 (56.0–68.0)	59.0 (53.0–66.0)	60.0 (54.0–67.3)	0.64
SBP, mmHg	140 (128–153)	145 (129–160)	138 (128–151)	0.55
DBP, mmHg	78.0 (70.3–85.0)	78.0 (71.3–85.8)	77.0 (71.0–84.0)	0.63
Blood tests				
Haemoglobin, g/dL	14.1 (13.1–14.9)	14.0 (13.2–14.9)	14.0 (12.9–14.8)	0.24
Sodium, mmol/L	139 (137–141)	140 (138–141)	139 (138–141)	0.48
Potassium, mmol/L	4.3 (4.1–4.6)	4.3 (4.0–4.6)	4.3 (4.1–4.5)	0.78
eGFR, mL/min/1.73 m^2	80 (68–88)	73 (61–82)	70 (57–82)	**<0.001**
Electrocardiography				
QRS duration (ms)	92.0 (84.8–105)	90.0 (82.0–102)	94.0 (82.5–114)	0.17
Echocardiography				
LVEDVI, mL/m^2	40.5 (35.6–48.8)	43.2 (36.4–49.7)	41.0 (34.6–47.8)	0.60
LVEF, %	63.5 (58.0–68.0)	63.1 (57.9–66.2)	62.0 (57.1–66.4)	0.14
LVMI, g/m^2	90.7 (77.9–103)	97.2 (84.2–114)	96.5 (80.1–114)	0.06
LAVI, mL/m^2	31 (26–36)	31 (27–37)	30 (25–37)	0.89
E:A ratio	0.8 (0.7–1.0)	0.9 (0.7–1.0)	0.8 (0.6–1.0)	0.57
E:e′ ratio	9.3 (7.5–11.3)	9.5 (7.6–11.6)	8.9 (7.7–11.6)	0.44
TAPSE	22.9 (18.0–26.9)	22.6 (17.5–26.6)	20.4 (16.4–25.6)	**0.015**
Blood biomarkers				
NT-proBNP, ng/L	190 (133–296)	214 (133–357)	253 (142–395)	**0.004**
Hs-TnT, ng/L	11.4 (7.9–15.1)	13.2 (8.8–18.7)	13.9 (9.7–19.9)	**<0.001**
PICP, µg/L	76.5 (62.4–93.3)	81.0 (66.7–95.5)	82.7 (67.1–105)	**0.001**
PIIINP, µg/L	3.6 (2.8–4.7)	3.9 (3.1–4.8)	4.4 (3.4–5.5)	**<0.001**
CITP, µg/L	3.1 (2.4–3.9)	3.5 (2.8–4.5)	4.8 (3.8–6.4)	**<0.001**
MMP-1, µg/L	18.8 (13.6–24.2)	9.8 (7.6–12.8)	6.0 (4.3–8.2)	**<0.001**

Values are expressed as median (interquartile range) and categorical variables as numbers and percentages.
A, late (atrial) mitral flow velocity; ACE, angiotensin-converting enzyme; BMI, body mass index; CCL, collagen cross-linking; CITP, collagen type I C-terminal telopeptide; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; E, early mitral flow velocity; e′, early diastolic tissue velocity; eGFR, estimated glomerular filtration rate; HR, heart rate; Hs-TnT, high-sensitivity troponin T; LAVI, left atrial volume index; LV, left ventricular; LVEDVI, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; MMP-1, matrix metalloproteinase-1; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PICP, procollagen type I C-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; SBP, systolic blood pressure; TAPSE, tricuspid annular plane systolic excursion.

were driven by an increase in CITP rather than MMP-1, which was unaffected by spironolactone (*Table 2*). Increases in CITP:MMP-1 with spironolactone were mainly observed in the first and second tertiles of CITP:MMP-1 (online supplementary *Figure S2* and *Table 2*). However, CITP values after treatment with spironolactone in the first tertile (highest CCL) were still lower than CITP in the third tertile (lowest CCL) (3.62, 95% CI 2.74–4.64 vs. 4.23, 95% CI 3.47–6.74 µg/L; $p < 0.001$).

Table 2 Spironolactone versus control differences in echocardiographic variables and biomarkers after 1 month and at the end of trial (9 months) in patients categorized according to baseline CITP:MMP-1 tertiles (first = highest and third = lowest collagen cross-linking)

	Difference vs. control						Within tertile interaction *p*-value Ctr vs. Spiro	Across-tertiles interaction *p*-value
	1 month			9 month				
	Difference	95% CI	*p*-value	Difference	95% CI	*p*-value		
Echocardiographic variables								
LAVI, mL/m^2								
First tertile	1.18	0.12 to 2.24	**0.029***	0.05	−1.58 to 1.67	0.96	0.19	**0.005***
Second tertile	−1.62	−2.70 to −0.53	**0.004***	−0.40	−2.29 to 1.49	0.68	0.12	
Third tertile	−1.77	−2.94 to −0.59	**0.003***	−2.52	−4.46 to −0.58	**0.011***	**0.008***	
E wave, cm/s								
First tertile	−0.06	−0.09 to −0.04	**<0.001***	−0.03	−0.07 to 0.01	0.12	**<0.001***	0.88
Second tertile	−0.06	−0.09 to −0.02	**0.001***	−0.07	−0.12 to −0.02	**0.004***	**0.011***	
Third tertile	−0.06	−0.09 to −0.03	**<0.001***	−0.06	−0.11 to −0.01	**0.023***	**0.017***	
E:A ratio								
First tertile	−0.09	−0.16 to −0.02	**0.016***	−0.03	−0.11 to 0.05	0.45	0.08	0.40
Second tertile	−0.13	−0.21 to −0.04	**0.003***	−0.07	−0.15 to 0.01	0.11	**0.009***	
Third tertile	−0.08	−0.13 to −0.03	**0.001***	−0.12	−0.24 to −0.01	**0.040**	**0.019***	
LVMI, g/m^2								
First tertile	−2.01	−5.12 to 1.11	0.21	−3.02	−7.20 to 1.15	0.16	0.33	0.86
Second tertile	−1.44	−5.36 to 2.48	0.47	−1.43	−5.50 to 2.64	0.49	0.69	
Third tertile	0.57	−3.90 to 5.04	0.80	−0.85	−4.79 to 3.08	0.67	0.83	
LVEDVI, mL/m^2								
First tertile	1.04	−0.42 to 2.51	0.16	0.63	−1.31 to 2.56	0.53	0.39	0.82
Second tertile	−0.07	−2.09 to 1.96	0.95	−0.36	−2.33 to 1.61	0.72	0.95	
Third tertile	0.22	−1.95 to 2.39	0.85	−0.17	−1.52 to 1.18	0.80	0.94	
LVEF, %								
First tertile	−0.25	−2.44 to 1.93	0.82	1.57	−0.36 to 3.50	0.11	0.24	0.62
Second tertile	−0.91	−2.22 to 0.40	0.18	1.86	0.12 to 3.60	0.036	**0.039**	
Third tertile	0.02	−1.30 to 1.34	0.98	0.30	−1.41 to 2.01	0.73	0.87	
E:e′ ratio								
First tertile	−0.64	−1.42 to 0.15	0.11	−0.49	−1.29 to 0.30	0.22	0.30	0.14
Second tertile	0.69	0.16 to 1.23	**0.011***	−0.27	−1.14 to 0.60	0.55	**0.040**	
Third tertile	−0.55	−1.07 to −0.03	**0.040**	−0.32	−1.12 to 0.48	0.44	0.27	
TAPSE								
First tertile	1.51	−0.21 to 3.22	0.09	−0.08	−1.73 to 1.57	0.92	0.18	0.12
Second tertile	−1.16	−2.83 to 0.51	0.17	−1.52	−2.96 to −0.08	**0.040**	0.26	
Third tertile	−0.21	−1.33 to 0.91	0.72	0.65	−1.27 to 2.56	0.51	0.68	

Table 2 (Continued)

	Difference vs. control						Within tertile interaction *p*-value Ctr vs. Spiro	Across-tertiles interaction *p*-value
	1 month			9 month				
	Difference	95% CI	*p*-value	Difference	95% CI	*p*-value		
Biomarkers								
NT-proBNP (log_2), ng/L								
First tertile	−0.21	−0.43 to 0.01	0.06	−0.16	−0.41 to 0.08	0.20	0.06	0.09
Second tertile	−0.35	−0.52 to −0.17	**<0.001***	−0.09	−0.32 to 0.15	0.48	**<0.001***	
Third tertile	−0.47	−0.66 to −0.28	**<0.001***	−0.31	−0.59 to −0.04	**0.024***	**<0.001***	
Hs-TnT (log_2), ng/L								
First tertile	−0.07	−0.17 to 0.04	0.23	−0.03	−0.17 to 0.12	0.70	0.75	
Second tertile	0.08	−0.03 to 0.20	0.16	0.08	−0.07 to 0.23	0.29	0.99	0.75
Third tertile	0.05	−0.11 to 0.22	0.54	0.11	−0.07 to 0.29	0.23	0.32	
CITP:MMP-1 ratio (log_2)								
First tertile	0.18	0.08 to 0.27	**<0.001***	0.13	−0.05 to 0.30	0.16	**0.031**	0.19
Second tertile	0.09	−0.001 to 0.18	0.052	0.25	0.06 to 0.44	**0.011***	0.06	
Third tertile	−0.001	−0.17 to 0.16	0.99	−0.11	−0.29 to 0.08	0.27	0.51	
CITP (log_2), µg/L								
First tertile	0.16	0.07 to 0.24	**<0.001***	0.11	−0.04 to 0.27	0.15	**0.029**	0.11
Second tertile	0.06	−0.01 to 0.14	0.09	0.19	0.05 to 0.33	**0.008***	**0.015***	
Third tertile	0.02	−0.10 to 0.14	0.72	−0.06	−0.22 to 0.09	0.42	0.58	
MMP-1 (log_2), µg/L								
First tertile	−0.05	−0.15 to 0.05	0.31	−0.04	−0.17 to 0.10	0.58	0.94	0.84
Second tertile	0.02	−0.06 to 0.09	0.67	−0.02	−0.14 to 0.11	0.80	0.87	
Third tertile	0.09	−0.03 to 0.21	0.14	0.11	−0.01 to 0.24	0.08	0.71	
PICP, µg/L								
First tertile	−6.25	−11.2 to −1.29	**0.014***	−9.19	−15.5 to −2.85	**0.004***	**0.003***	0.91
Second tertile	−5.63	−11.0 to −0.26	**0.040**	−6.99	−13.3 to −0.64	**0.031**	**0.031**	
Third tertile	−4.93	−8.96 to −0.90	**0.016***	−6.32	−13.9 to 1.33	0.11	0.28	
PIIINP, µg/L								
First tertile	0.03	−0.35 to 0.41	0.89	−0.28	−0.81 to 0.24	0.29	0.55	0.52
Second tertile	−0.14	−0.60 to 0.32	0.56	0.02	−0.50 to 0.54	0.95	0.83	
Third tertile	0.008	−0.48 to 0.50	0.98	0.07	−0.47 to 0.61	0.81	0.58	

CITP, collagen type I C-terminal telopeptide; Hs-TnT, high-sensitivity troponin T; LAVI, left atrial volume index; LVEDVI, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; MMP-1, matrix metalloproteinase-1; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PICP, procollagen type I C-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; TAPSE, tricuspid annular plane systolic excursion.

Differences between spironolactone (Spiro) and control (Ctrl) were expressed as mean and 95% CI.

*Significant after multiple test correction [Benjamini and Hochberg (5% false discovery rate)].

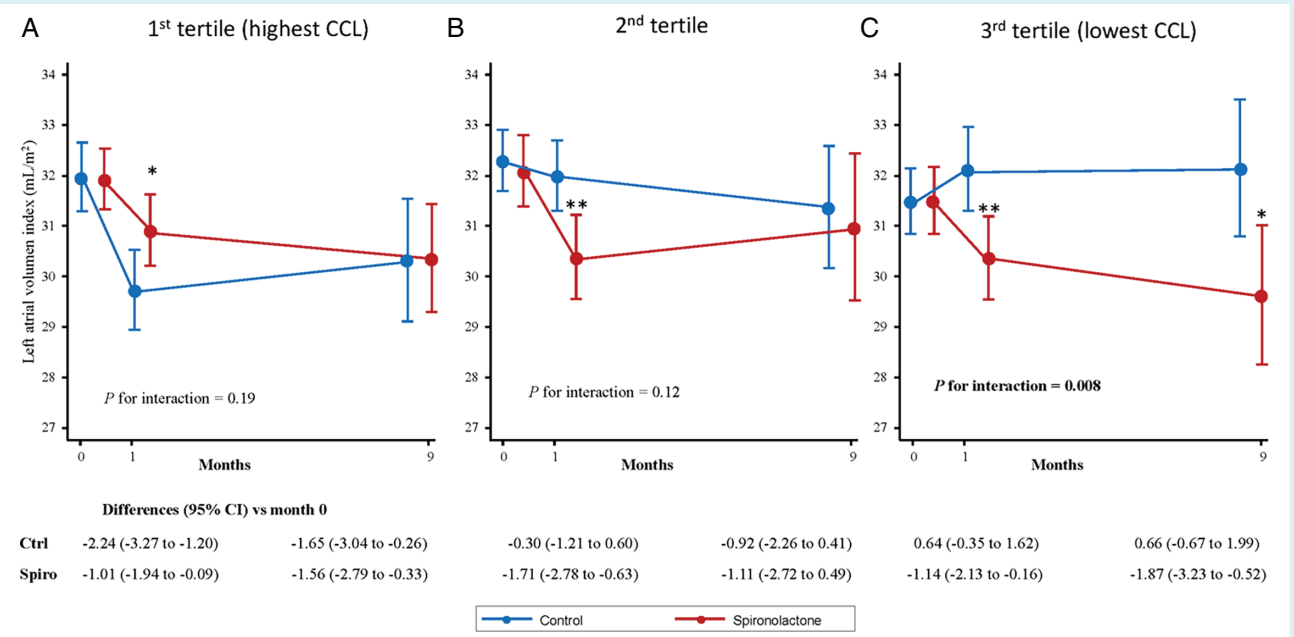


Figure 1 Left atrial volume index according to assigned treatment in patients with low (*A*: first tertile), medium (*B*: second tertile) and high (*C*: third tertile) collagen type I C-terminal telopeptide to matrix metalloproteinase-1 ratio (CITP:MMP-1) levels. Data are expressed as the predicted mean values (estimated marginal means of visits by group) and 95% confidence interval (CI) at baseline, visit 1 (1 month) and visit 2 (9 months) in patients treated with standard of care or spironolactone after linear mixed regression analysis with left atrial volume index as the dependent variable, and treatment, visit, their interaction term, and baseline estimated glomerular filtration rate and left atrial volume index values as fixed effects. Mean changes (95% CI) values versus baseline are shown at the bottom of each panel. *$p < 0.05$ spironolactone versus control, **$p < 0.01$ spironolactone versus control. CCL, collagen cross-linking.

Reductions in PICP, a marker of collagen synthesis, were similar across CITP:MMP-1 tertiles, although tended to be greater in the first tertile (highest CCL) (*Table 2*). Spironolactone did not reduce serum PIIINP in any CITP:MMP-1 tertiles (*Table 2*).

Discussion

To our knowledge this is the first randomized trial evaluating the influence of CCL (indirectly assessed by circulating biomarkers) on the effects of spironolactone in patients at risk of HF. Our main finding is that effects of spironolactone on LAVI and, to a lesser extent, on NT-proBNP, were more prominent in patients with-higher CITP:MMP-1 ratio (reflecting lower CCL). Effects appeared within 1 month and persisted to the end of the study only in patients with a low CCL (high CITP:MMP-1 ratio). The robustness of these findings is supported by the interaction analysis and adjustment for potential confounding factors (including eGFR).

Our findings extend previous observations in symptomatic patients with a clinical diagnosis of HFpEF to asymptomatic patients at risk of HF. In a sub-study of the Aldo-DHF trial, we showed that the effects of spironolactone on variables reflecting LV diastolic dysfunction were restricted to patients with higher CITP:MMP-1 (reflecting low CCL).[13] However, whereas in Aldo-DHF spironolactone treatment was associated with a decrease in the E:e′ ratio with no changes in LAVI,[15] in HOMAGE spironolactone improved LAVI without affecting the E:e′ ratio.[2] This discrepancy may be related to the different clinical characteristics of the respective cohorts: whereas baseline E:e′ ratio was higher in patients in Aldo-DHF compared to HOMAGE (12.8 vs. 9.3), baseline LAVI was higher in HOMAGE (30.9 vs. 28.0 mL/m^2). Nevertheless, it is remarkable that when stratifying patients according to baseline serum CITP:MMP-1, both in the Aldo-DHF[13] and HOMAGE trials, the beneficial effects on measures of LV diastolic function are mostly found in patients with low CCL.

Baseline LAVI was similar across CITP:MMP-1 tertiles. Several factors other than CCL may contribute to LA enlargement. Patients in the lowest CCL tertile had higher serum concentrations of markers of collagen synthesis (both PICP and PIIINP) which may indicate higher collagen deposition. NT-proBNP was also higher in these patients pointing to additional mechanisms leading to myocardial stress, for example an expanded circulating volume. Nevertheless, despite higher NT-proBNP and higher serum concentration of collagen synthesis biomarkers, treatment with spironolactone had a larger impact on LAVI in patients with low CCL compared to other groups.

Left atrial volume index may be considered a surrogate marker of increased LV filling pressures that may better reflect the cumulative effect of filling pressures over time than Doppler indices[3] and predict the risk of incident HF[16] and outcome in patients with HF with[17] or without a reduced LV ejection fraction.[18] LA enlargement

Table 3 Spironolactone versus control adjusted differences in left atrial volume index after 1 month and at the end of trial (9 months) in patients categorized according to baseline CITP:MMP-1 tertiles (first = highest and third = lowest collagen cross-linking)

	Difference vs. control						Within tertile interaction *p*-value Ctr vs. Spiro	Across-tertiles interaction *p*-value
	1 month			9 month				
	Difference	95% CI	*p*-value	Difference	95% CI	*p*-value		
Model 1								
First tertile	1.54	0.42 to 2.65	**0.007**	0.10	−1.58 to 1.77	0.91	0.07	**0.001**
Second tertile	−2.04	−3.15 to −0.93	**<0.001**	−0.40	−2.36 to 1.56	0.69	**0.021**	
Third tertile	−1.57	−2.78 to −0.35	**0.011**	−2.41	−4.39 to −0.43	**0.017**	**0.020**	
Model 2								
First tertile	1.39	0.28 to 2.50	0.014	0.26	−1.37 to 1.88	0.76	0.12	**0.001**
Second tertile	−2.20	−3.34 to −1.05	**<0.001**	−0.35	−2.30 to 1.61	0.73	**0.008**	
Third tertile	−1.70	−2.92 to −0.48	**0.006**	−2.49	−4.47 to −0.51	**0.014**	**0.018**	
Model 3								
First tertile	1.33	0.21 to 2.45	0.020	0.33	−1.29 to 1.96	0.69	0.17	**0.003**
Second tertile	−2.05	−3.18 to −0.93	**<0.001**	0.02	−1.95 to 2.00	0.98	**0.016**	
Third tertile	−1.64	−2.88 to −0.40	**0.009**	−2.07	−4.08 to −0.06	**0.044**	**0.039**	

CI, confidence interval; CITP, collagen type I C-terminal telopeptide; Ctr, control; eGFR, estimated glomerular filtration rate; hs-TnT, high-sensitivity troponin T; LAVI, left atrial volume index; MMP-1, matrix metalloproteinase-1; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PICP, procollagen type I C-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; Spiro, spironolactone; TAPSE, tricuspid annular plane systolic excursion.
Model 1: Baseline body mass index, coronary artery bypass graft, eGFR, TAPSE, and LAVI.
Model 2: Baseline body mass index, coronary artery bypass graft, eGFR, TAPSE, LAVI, hs-TnT and NT-proBNP.
Model 3: Baseline body mass index, coronary artery bypass graft, eGFR, TAPSE, LAVI, hs-TnT, NT-proBNP, PICP, and PIIINP.

is also a risk factor for AF,[4] especially for HFpEF.[19] Reciprocally, LAVI will increase with the onset of AF.[20] Atrial fibrosis is a major pathophysiological contributor to AF and has been linked to AF recurrence and resistance to therapy.[21] Interestingly, an increase in CCL has been observed in the LA appendage of patients with AF.[22] We have previously reported that patients with HF and serum concentrations of collagen biomarkers reflecting high collagen type I synthesis and high CCL have a high incidence and prevalence of AF.[23] Altogether, these data suggest that decreasing LAVI before the onset of HF might not only delay the onset of clinical HF but also of AF, one of its potential precipitants.

Increased plasma NT-proBNP is associated with LAVI in HFpEF[5,24] and this was also confirmed in this study evaluating patients at risk of HF. The reduction in NT-proBNP with spironolactone observed in the overall trial[2] was most prominent in patients with the highest CITP:MMP-1 ratio, reinforcing the hypothesis that patients with low CCL might be those who benefit the most from the disease-modifying effects of spironolactone.

Both the extent of collagen formation and the quality of the fibres (i.e. collagen type or degree of CCL) contribute to the detrimental impact of myocardial fibrosis. The ability of mineralocorticoid receptor antagonists (MRA) to reduce biomarkers of collagen synthesis in patients with HF[13,25–27] or patients at risk of HF[2,28] is well established. In the current study, spironolactone reduced PICP across CITP:MMP-1 tertiles but in the Aldo-DHF trial no change in PICP was observed in those with a low CITP:MMP-1 ratio (high CCL).[13] This difference may be related to a more severe fibrosis phenotype in Aldo-DHF, as assessed by PICP values, where baseline median value was 110 μg/L (interquartile range: 92–136 μg/L) compared to a median value of only 81 ng/ml (interquartile range: 65–97 μg/L) in HOMAGE.

From a mechanistic point of view, the anti-fibrotic effects of spironolactone might be mostly due to inhibiting excessive synthesis of new collagen molecules, as PICP reflects the processing of procollagen into collagen. However, collagen fibre formation requires a subsequent step of cross-linking of collagen molecules (i.e. CCL) for fibre stabilization, a process increasing stiffness and resistance to degradation.[6,9] Increased CCL is associated with higher LV filling pressures both in patients with HFpEF[7] or AF.[22] The current findings suggest that only reducing the synthesis of collagen (as assessed by PICP) may not be associated with beneficial atrial remodelling when CCL is high. Therefore, it is tempting to speculate that spironolactone may affect upstream collagen synthesis, but is less effective or ineffective on CCL.

In this context, cardiac CCL emerges as a potential therapeutic target in patients at risk of HF. We have previously reported that HF patients with high CCL have a higher risk of hospitalizations for HF,[10] and that a combination of increased collagen type I synthesis and high CCL portends a worse outcome in terms of HF hospitalization and/or cardiovascular death[29] and a higher risk of AF.[23] Some treatments for HF might interfere with CCL. In patients with hypertensive HF, torasemide, a loop diuretic, reduced myocardial collagen type I deposition and CCL as well as the expression of lysyl oxidase, an important enzyme in the cross-linking process.[30] Further clinical research is required to investigate their effects and potential interactions with MRA.

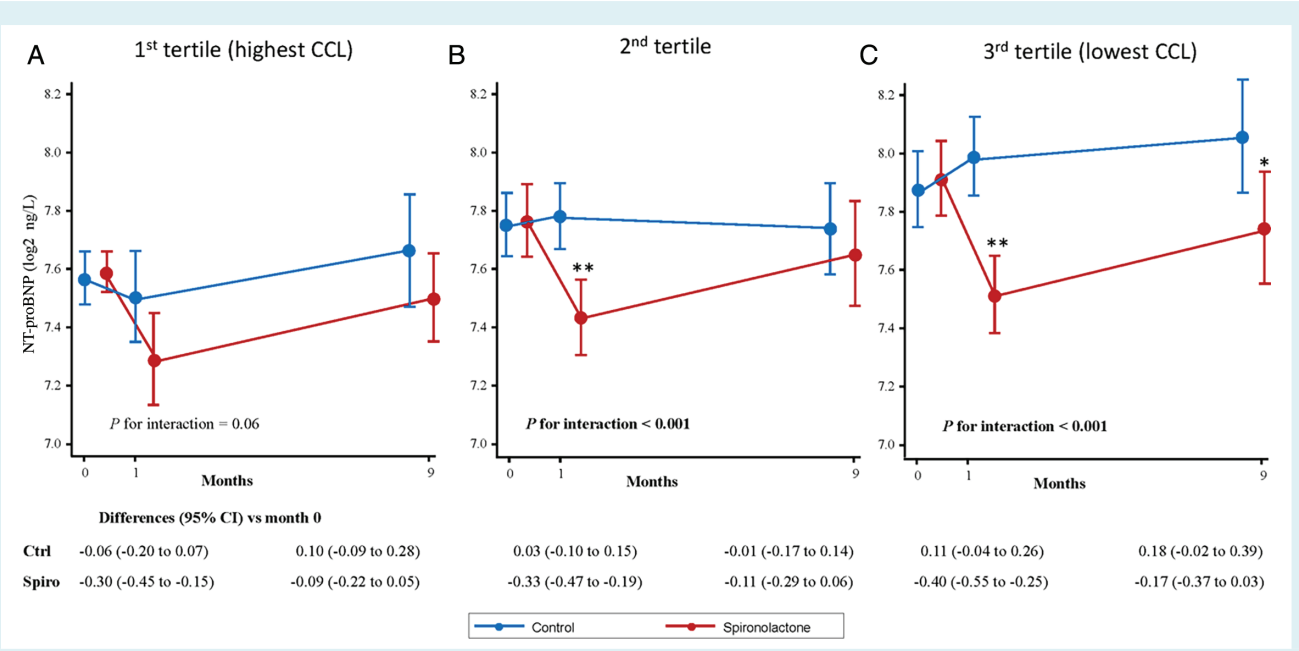


Figure 2 N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels (log2) according to assigned treatment in patients with low (*A*: first tertile), medium (*B*: second tertile) and high (*C*: third tertile) collagen type I C-terminal telopeptide to matrix metalloproteinase-1 ratio (CITP:MMP-1) levels. Data are expressed as the predicted mean values (estimated marginal means of visits by group) and 95% confidence interval (CI) at baseline, visit 1 (1 month) and visit 2 (9 months) in patients treated with standard of care or spironolactone after linear mixed regression analysis with NT-proBNP as the dependent variable, and treatment, visit, their interaction term, and baseline estimated glomerular filtration rate and NT-proBNP values as fixed effects. Mean changes (95% CI) values versus baseline are shown at the bottom of each panel. *$p < 0.05$ spironolactone versus control, **$p < 0.01$ spironolactone versus control. CCL, collagen cross-linking.

Some limitations need to be acknowledged. This is a post-hoc analysis that should be considered hypothesis-generating rather than conclusive evidence. CCL was not directly assessed in cardiac tissue; we used surrogate serum biomarkers which are not cardiac-specific. In this regard, although hepatic, lung and auto-immune diseases were exclusion criteria for recruitment, we cannot exclude that other occult conditions may have affected systemic circulating CITP:MMP-1 levels. Our patients were at risk of HF but not clinically symptomatic and did not have severe LV diastolic dysfunction. However, patients in HOMAGE were very similar to many patients enrolled in trials of HFpEF in terms of LA volume and plasma concentrations of NT-proBNP, and the presence of occult HFpEF cannot be excluded. Many of the patients enrolled in the HOMAGE trial would fulfil a new 'universal' definition of HF proposed by some experts.[1] Phenotyping of HOMAGE patients by additional parameters related not only to ventricular and atrial contractility and stiffness, but also to collagen synthesis, fibrosis and long-term prognosis such as speckle tracking echocardiography, was not available. Finally, HOMAGE was a relatively short-term mechanistic trial rather than a long-term outcome trial and therefore we were not able to evaluate the effects of spironolactone treatment on new-onset HF, morbidity and mortality. Large prospective longitudinal multicentre outcome trials would be necessary to validate our hypothesis-generating findings.

In conclusion, we show for the first time that, in patients at risk of HF with blood markers consistent with low CCL, spironolactone exerts greater effects on LA remodelling (*Graphical Abstract*). Biochemical phenotyping of myocardial CCL might facilitate a precision medicine approach to the prevention of HF by identifying patients in whom spironolactone might delay or prevent the development of atrial dilatation and progression to clinically overt HF.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Acknowledgements

The authors want to thank Sonia Martínez and Laura Martínez for their technical support.

Funding

This work was supported by the European Commission HOMAGE project (grant 305507); JD and AG are supported by the Ministry of Science and Innovation, Spain (Instituto de Salud Carlos III: CB16/11/00483 and PI18/01469 co-financed by FEDER funds). MH is supported by a Kootstra Talented Post-doc Fellowship (Netherlands). JGFC and PP are supported by the British Heart Foundation Centre of Research Excellence (grant number RE/18/6/34217). PPREMED (URL: http://www.appremed.orf) received a non-binding grant from Omron Healthcare Co., Ltd., Kyoto, Japan.

Conflict of interest: Research support and personal honoraria for committees and lectures: S.H.: Pfizer, Zeiss, Sanquin, AstraZeneca and Novartis. J.G.F.C.: Amgen, Bristol Myers Squibb, Medtronic, Pharmacosmos, Servier and Vifor Pharma. F.Z.: Applied Therapeutics, Bayer, Boehringer, Boston Scientific, Novartis, Janssen and CVRx, AstraZeneca, Vifor Fresenius, Cardior, Cereno pharmaceutical and Merck, stock options at G3Pharmaceutical, and being the founder of CardioRenal and CVCT. All other authors have nothing to disclose.

References

1. Cleland JGF, Pfeffer MA, Clark AL, Januzzi JL, McMurray JJV, Mueller C, et al. The struggle towards a universal definition of heart failure – how to proceed? *Eur Heart J*. 2021;**42**:2331–43.
2. Cleland JGF, Ferreira JP, Mariottoni B, Pellicori P, Cuthbert J, Verdonschot JAJ, et al. 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) randomized clinical trial. *Eur Heart J*. 2021;**42**:684–96.
3. Pritchett AM, Mahoney DW, Jacobsen SJ, Rodeheffer RJ, Karon BL, Redfield MM. Diastolic dysfunction and left atrial volume: a population-based study. *J Am Coll Cardiol*. 2005;**45**:87–92.
4. Hoit BD. Left atrial size and function: role in prognosis. *J Am Coll Cardiol*. 2016;**63**:493–505.
5. Pellicori P, Zhang J, Lukaschuk E, Joseph AC, Bourantas CV, Loh H, et al. Left atrial function measured by cardiac magnetic resonance imaging in patients with heart failure: clinical associations and prognostic value. *Eur Heart J*. 2015;**36**:733–43.
6. Zile MR, Baicu CF, Ikonomidis JS, Stroud RE, Nietert PJ, Bradshaw AD, et al. Myocardial stiffness in patients with heart failure and a preserved ejection fraction contributions of collagen and titin. *Circulation*. 2015;**131**:1247–59.
7. Kasner M, Westermann D, Lopez B, Gaub R, Escher F, Kühl U, et al. Diastolic tissue doppler indexes correlate with the degree of collagen expression and cross-linking in heart failure and normal ejection fraction. *J Am Coll Cardiol*. 2011;**57**:977–85.
8. Díez J, González A, Kovacic JC. Myocardial interstitial fibrosis in nonischemic heart disease, part 3/4: JACC focus seminar. *J Am Coll Cardiol*. 2020;**75**:2204–18.
9. González A, López B, Ravassa S, San José G, Díez J. The complex dynamics of myocardial interstitial fibrosis in heart failure. Focus on collagen cross-linking. *Biochim Biophys Acta Mol Cell Res*. 2019;**1866**:1421–32.
10. López B, Ravassa S, González A, Zubillaga E, Bonavila C, Bergés M, et al. Myocardial collagen cross-linking is associated with heart failure hospitalization in patients with hypertensive heart failure. *J Am Coll Cardiol*. 2016;**67**:251–60.
11. Van Der Slot-Verhoeven AJ, Van Dura EA, Attema J, Blauw B, DeGroot J, Huizinga TWJ, et al. The type of collagen cross-link determines the reversibility of experimental skin fibrosis. *Biochim Biophys Acta*. 2005;**1740**:60–7.
12. Philp CJ, Siebeke I, Clements D, Miller S, Habgood A, John AE, et al. Extracellular matrix cross-linking enhances fibroblast growth and protects against matrix proteolysis in lung fibrosis. *Am J Respir Cell Mol Biol*. 2018;**58**:594–603.
13. Ravassa S, Trippel T, Bach D, Bachran D, González A, López B, et al. Biomarker-based phenotyping of myocardial fibrosis identifies patients with heart failure with preserved ejection fraction resistant to the beneficial effects of spironolactone: results from the Aldo-DHF trial. *Eur J Heart Fail*. 2018;**20**:1290–9.
14. Pellicori P, Ferreira JP, Mariottoni B, Brunner-La Rocca HP, Ahmed FZ, Verdonschot J, et al. Effects of spironolactone on serum markers of fibrosis in people at high risk of developing heart failure: rationale, design and baseline characteristics of a proof-of-concept, randomised, precision-medicine, prevention trial. The Heart OMics in AGing (HOMAGE) trial. *Eur J Heart Fail*. 2020;**22**:1711–23.
15. Edelmann F, Wachter R, Schmidt AG, Kraigher-Krainer E, Colantonio C, Kamke W, et al.; Aldo-DHF Investigators. Effect of spironolactone on diastolic function and exercise capacity in patients with heart failure with preserved ejection fraction: the Aldo-DHF randomized controlled trial. *JAMA*. 2013;**309**:781–91.
16. Hiemstra YL, Debonnaire P, van Zwet EW, Bootsma M, Schalij MJ, Bax JJ, et al. Development of and progression of overt heart failure in nonobstructive hypertrophic cardiomyopathy. *Am J Cardiol*. 2018;**122**:656–62.
17. Gulati A, Ismail TF, Jabbour A, Ismail NA, Morarji K, Ali A, et al. Clinical utility and prognostic value of left atrial volume assessment by cardiovascular magnetic resonance in non-ischaemic dilated cardiomyopathy. *Eur J Heart Fail*. 2013;**15**:660–70.
18. Shah AM, Claggett B, Sweitzer NK, Shah SJ, Deswal A, Anand IS, et al. Prognostic importance of changes in cardiac structure and function in heart failure with preserved ejection fraction and the impact of spironolactone. *Circ Heart Fail*. 2015;**8**:1052–8.
19. Lam CSP, Rienstra M, Tay WT, Liu LCY, Hummel YM, van der Meer P, et al. Atrial fibrillation in heart failure with preserved ejection fraction: association with exercise capacity, left ventricular filling pressures, natriuretic peptides, and left atrial volume. *JACC Heart Fail*. 2017;**5**:92–8.
20. Njoku A, Kannabhiran M, Arora R, Reddy P, Gopinathannair R, Lakkireddy D, et al. Left atrial volume predicts atrial fibrillation recurrence after radiofrequency ablation: a meta-analysis. *Europace*. 2018;**20**:33–42.
21. Nattel S. Molecular and cellular mechanisms of atrial fibrosis in atrial fibrillation. *JACC Clin Electrophysiol*. 2017;**3**:425–35.
22. Adam O, Theobald K, Lavall D, Grube M, Kroemer HK, Ameling S, et al. Increased lysyl oxidase expression and collagen cross-linking during atrial fibrillation. *J Mol Cell Cardiol*. 2011;**50**:678–85.
23. Ravassa S, Ballesteros G, López B, Ramos P, Bragard J, González A, et al. Combination of circulating type I collagen-related biomarkers is associated with atrial fibrillation. *J Am Coll Cardiol*. 2019;**73**:1398–410.
24. Kim H, Jun DW, Cho YK, Nam CW, Han SW, Hur SH, et al. The correlation of left atrial volume index to the level of N-terminal pro-BNP in heart failure with a preserved ejection fraction. *Echocardiography*. 2008;**25**:961–7.
25. Zannad F, Alla F, Dousset B, Perez A, Pitt B. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insights from the Randomized Aldactone Evaluation Study (RALES). *Circulation*. 2000;**102**:2700–6.
26. Iraqi W, Rossignol P, Angioi M, Fay R, Nuée J, Ketelslegers JM, et al. Extracellular cardiac matrix biomarkers in patients with acute myocardial infarction complicated by left ventricular dysfunction and heart failure: insights from the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) study. *Circulation*. 2009;**119**:2471–9.
27. Stienen S, Ferreira JP, Pitt B, Cleland JG, Pellicori P, Girerd N, et al. Eplerenone prevents an increase in serum carboxy-terminal propeptide of procollagen type I after myocardial infarction complicated by left ventricular dysfunction and/or heart failure. *Eur J Heart Fail*. 2020;**22**:901–3.
28. Ferreira JP, Rossignol P, Pizard A, Machu JL, Collier T, Girerd N, et al. Potential spironolactone effects on collagen metabolism biomarkers in patients with uncontrolled blood pressure. *Heart*. 2019;**105**:307–14.
29. Ravassaa S, López B, Querejeta R, Echegaray K, José GS, Moreno MU, et al. Phenotyping of myocardial fibrosis in hypertensive patients with heart failure. Influence on clinical outcome. *J Hypertens*. 2017;**35**:853–61.
30. López B, Querejeta R, González A, Beaumont J, Larman M, Díez J. Impact of treatment on myocardial lysyl oxidase expression and collagen cross-linking in patients with heart failure. *Hypertension*. 2009;**53**:236–42.