

SUPPLEMENTAL MATERIAL

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

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Supplemental Table 1. Demographic, clinical and biochemical characteristics at baseline in patients classified according to the CITP:MMP-1 ratio tertiles (1st = highest and 3rd = lowest CCL) and treatment

	1 st tertile (Highest CCL)			2 nd tertile			3 rd tertile (Lowest CCL)		
	Placebo (n=75)	Spirolactone (n=96)	<i>P</i>	Placebo (n=96)	Spirolactone (n=76)	<i>P</i>	Placebo (n=84)	Spirolactone (n=88)	<i>P</i>
CITP:MMP-1 ratio	0.6 (0.4-0.8)	0.6 (0.4-0.8)	0.80	1.3 (1.1-1.6)	1.2 (1.1-1.5)	0.05	2.9 (2.0-4.3)	2.5 (2.1-3.4)	0.15
<u>Demographics</u>									
Age, years	71 (67-77)	72 (68-77)	0.61	72 (68-77)	73 (69-77)	0.73	73 (68-79)	72 (68-79)	0.78
Women, n (%)	16 (21.3)	25 (26.0)	0.47	28 (29.2)	15 (19.7)	0.16	28 (33.3)	19 (21.6)	0.08
Current smoker, n(%)	5 (6.7)	8 (8.3)	0.71	9 (9.4)	4 (5.3)	0.32	4 (4.8)	11 (12.5)	0.07
<u>Prior Medical History, n (%)</u>									
Hypertension	50 (66.7)	78 (81.3)	0.03	79 (82.3)	61 (80.3)	0.73	65 (77.4)	70 (79.5)	0.73
Diabetes mellitus	28 (37.3)	42 (43.8)	0.40	42 (43.8)	31 (40.8)	0.70	32 (38.1)	33 (37.5)	0.94
Coronary artery disease	60 (80.0)	66 (68.8)	0.10	65 (67.7)	54 (71.1)	0.64	59 (70.2)	66 (75.0)	0.48
Myocardial infarction	33 (44.0)	39 (40.6)	0.66	40 (41.7)	35 (46.1)	0.56	28 (33.3)	37 (42.0)	0.24
Percutaneous Coronary Intervention	50 (66.7)	46 (47.9)	0.01	49 (51.0)	36 (47.4)	0.63	34 (40.5)	46 (52.3)	0.12
Coronary Artery Bypass Graft	19 (25.3)	19 (19.8)	0.39	25 (26.0)	18 (23.7)	0.72	25 (29.8)	29 (33.0)	0.65
Stroke/Transient Ischemic Attack	2 (2.7)	6 (6.3)	0.47	7 (7.3)	2 (2.6)	0.30	3 (3.6)	7 (8.0)	0.33
COPD	5 (6.7)	10 (10.4)	0.39	3 (3.1)	4 (5.3)	0.70	4 (4.8)	6 (6.8)	0.75
<u>Baseline medications, n (%)</u>									
ACE inhibitor	45 (60.0)	48 (50.0)	0.19	49 (51.0)	40 (52.6)	0.84	43 (51.2)	44 (50.0)	0.88
Angiotensin Receptor Blockers	13 (17.3)	29 (30.2)	0.05	31 (32.3)	20 (26.3)	0.39	27 (32.1)	23 (26.1)	0.39
Beta-blocker	46 (61.3)	65 (67.7)	0.39	69 (71.9)	52 (68.4)	0.62	62 (73.8)	65 (73.9)	0.99
Thiazide Diuretics	8 (10.7)	20 (20.8)	0.08	23 (24.0)	16 (21.1)	0.65	10 (11.9)	10 (11.4)	0.91
Calcium channel blocker	14 (18.7)	19 (19.8)	0.85	24 (25.0)	19 (25.0)	>0.99	13 (15.5)	19 (21.6)	0.30
Lipid-lowering drug	69 (92.0)	76 (79.2)	0.02	74 (77.1)	63 (82.9)	0.35	68 (81.0)	74 (84.1)	0.59
Aspirin	55 (73.3)	68 (70.8)	0.72	66 (68.8)	55 (72.4)	0.61	55 (65.5)	68 (77.3)	0.09
Any antiplatelet (including aspirin)	58 (77.3)	75 (78.1)	0.90	75 (78.1)	58 (76.3)	0.78	66 (78.6)	71 (80.7)	0.73

Physical examination

BMI, kg/m ²	27.0 (25.2-31.1)	28.3 (25.6-31.2)	0.69	28.2 (25.9-30.5)	28.2 (24.9-31.3)	0.97	28.0 (25.1-33.2)	29.4 (26.6-32.3)	0.55
HR, beats/min	62.5 (56.0-67.0)	63.0 (56.0-71.0)	0.45	61.0 (53.0-68.0)	58.0 (54.0-66.0)	0.43	61.0 (54.0-68.0)	60.0 (55.0-67.0)	0.64
SBP, mmHg	136 (128-153)	142 (128-153)	0.74	146 (129-162)	141 (130-159)	0.56	138 (128-151)	140 (125-151)	0.91
DBP, mmHg	78 (72-8)	77 (70-85)	0.33	77 (71-85)	80 (73-86)	0.47	76 (71-83)	78 (71-85)	0.43

Blood tests

Hemoglobin, g/dL	14.2 (13.1-15.0)	14.0 (13.1-14.7)	0.19	14.1 (13.5-14.9)	14.0 (12.9-15.2)	0.48	13.9 (12.5-14.7)	14.0 (13.1-14.9)	0.42
Sodium, mmol/L	139 (138-141)	139 (137-141)	0.81	140 (138-141)	140 (138-142)	0.86	139 (138-141)	140 (138-141)	0.84
Potassium, mmol/L	4.4 (4.1-4.5)	4.3 (4.0-4.6)	0.62	4.3 (4.1-4.6)	4.3 (4.0-4.6)	0.62	4.3 (4.1-4.6)	4.3 (4.1-4.5)	0.19
eGFR, mL/min/1.73 m ²	76 (65-88)	83 (69-89)	0.20	73 (61-80)	74 (60-87)	0.61	72 (54-83)	69 (61-82)	0.78

Echocardiography

LVEDVi, mL/m ²	44.8 (36.3-50.6)	38.1 (34.4-46.5)	0.20	43.4 (35.8-49.8)	43.2 (36.6-49.7)	0.57	40.6 (36.8-47.2)	41.6 (34.0-48.7)	0.95
LVEF, %	62.8 (55.9-65.9)	63.9 (58.8-68.6)	0.10	63.3 (56.2-66.0)	62.8 (59.2-66.3)	0.47	63.5 (57.5-67.8)	61.4 (56.3-65.5)	0.21
LVMI, g/m ²	89.5 (77.5-110)	91.7 (78.0-103)	0.91	99.1 (84.2-114)	93.5 (83.4-114)	0.79	94.1 (78.8-113)	97.7 (81.3-118)	0.85
LAVI, mL/m²	31 (26-37)	31(26-35)	0.48	32 (27-37)	30 (26-35)	0.24	29 (24-37)	30 (25-36)	0.84
E wave, m/s	0.6 (0.5-0.8)	0.7 (0.5-0.8)	0.43	0.7 (0.6-0.8)	0.7 (0.6-0.8)	0.82	0.7 (0.6-0.8)	0.7 (0.5-0.8)	0.63
E/A ratio	0.9 (0.7-1.0)	0.8 (0.7-1.0)	0.31	0.8 (0.7-1.0)	0.9 (0.7-1.1)	0.59	0.8 (0.7-0.9)	0.8 (0.6-1.1)	0.56
E/e' ratio	9.3 (7.2-11.8)	9.3 (7.6-11.2)	0.51	9.6 (7.8-12.2)	9.5 (7.3-11.2)	0.37	9.0 (7.4-11.7)	9.0 (8.0-11.7)	0.77
TAPSE	22.4 (15.7-26.8)	23.0 (19.1-27.4)	0.48	22.8 (17.7-27.0)	22.2 (17.5-25.0)	0.40	20.6 (16.4-25.8)	19.7 (15.8-24.2)	0.38

Blood biomarkers

NT-proBNP, ng/L	203 (128-293)	176 (135-313)	0.67	224 (137-330)	206 (125-412)	0.86	226 (128-386)	270 (146-423)	0.31
Hs-TnT, ng/L	11.4 (8.7-14.8)	11.3 (7.4-15.7)	0.69	13.6 (8.7-19.4)	13.2 (8.9-17.8)	0.49	13.5 (9.8-20.7)	14.0 (9.4-19.7)	0.72
PICP, µg/L	75.1 (62.8-92.2)	78.3 (61.8-95.3)	0.34	81.4 (67.0-95.5)	79.8 (63.1-95.8)	0.96	83.1 (72.7-116)	80.4 (64.1-103)	0.05
PIIINP, µg/L	3.4 (2.7-4.9)	3.6 (2.9-4.5)	0.89	3.8 (3.1-4.8)	3.9 (3.4-4.8)	0.55	4.6 (3.5-6.0)	4.2 (3.3-5.1)	0.04
CITP, µg/L	3.1 (2.5-3.7)	3.0 (2.4-4.1)	0.70	3.7 (3.0-4.7)	3.3 (2.7-4.1)	0.23	4.9 (3.8-6.5)	4.7 (3.7-6.3)	0.71
MMP-1, µg/L	19.4 (13.6-23.2)	18.6 (13.7-25.4)	0.93	10.0 (7.7-13.0)	9.3 (7.4-12.8)	0.93	6.1 (3.7-8.3)	6.0 (4.4-8.1)	0.34

CITP means collagen type I C-terminal telopeptide; MMP-1, matrix metalloproteinase-1; COPD, chronic obstructive pulmonary disease; ACE, angiotensin converting enzyme; NYHA, New York Heart Association; BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure;

eGFR, estimated glomerular filtration rate; LVEDVi, left ventricular (LV) end-diastolic volume index; LVEF, LV ejection fraction; LVMI, LV mass index; LAVI, left atrial volume index; E, early mitral flow velocity; A, late (atrial) mitral flow velocity; e', early diastolic tissue velocity; TAPSE: tricuspid annular plane systolic excursion; NT-proBNP; amino-terminal pro-brain natriuretic peptide; Hs-TnT, high sensitivity troponin T; PICP, procollagen type I C-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; C1P, collagen type I C-terminal telopeptide; MMP-1, matrix metalloproteinase.1 . Values are expressed as median (interquartile range) and categorical variables as numbers and percentages. Values are expressed as median (interquartile range) and categorical variables as numbers and percentages.

Supplemental Table 2. Spironolactone versus control differences in clinical variables at the end of trial in patients categorized according to the CITP:MMP-1 ratio tertiles (1st = highest and 3rd = lowest CCL)

	Difference vs control						<i>Within tertile interaction P Ctr vs Spiro</i>	<i>Across-tertiles interaction P</i>
	1 month			9 months				
	Difference	95% CI	<i>P</i>	Difference	95% CI	<i>P</i>		
BMI, kg/m²								
1 st tertile	-0.15	-0.26 to -0.04	0.006	-0.27	-0.57 to 0.03	0.08	0.07	
2 nd tertile	-0.20	-0.32 to -0.08	0.002	-0.16	-0.41 to 0.09	0.22	0.006	0.39
3 rd tertile	-0.08	-0.22 to 0.06	0.25	0.14	-0.21 to 0.48	0.44	0.29	
Heart Rate, bpm								
1 st tertile	1.08	-0.33 to 2.50	0.13	0.48	-1.89 to 2.85	0.69	0.54	
2 nd tertile	1.60	0.04 to 3.16	0.05	-0.73	-2.97 to 1.51	0.52	0.09	0.74
3 rd tertile	0.24	-1.50 to 1.97	0.79	0.22	-2.52 to 2.97	0.87	0.94	
SBP, mm Hg								
1 st tertile	-3.29	-6.95 to 0.38	0.079	-6.18	-11.7 to -0.63	0.029	0.09	
2 nd tertile	-11.9	-15.3 to -8.41	<0.001	-11.9	-17.8 to -6.00	<0.001	<0.001	0.09
3 rd tertile	-4.72	-8.13 to -1.32	0.007	-11.0	-15.9 to -6.14	0.002	<0.001	
DBP, mm Hg								
1 st tertile	-0.36	-2.25 to 1.54	0.71	-2.30	-5.35 to 0.75	0.14	0.42	
2 nd tertile	-2.63	-4.61 to -0.66	0.009	-2.89	-6.06 to 0.28	0.07	0.07	0.49
3 rd tertile	-2.57	-4.27 to -0.88	0.003	-3.06	-5.61 to -0.52	0.018	0.022	
Haemoglobin, g/dL								
1 st tertile	0.13	0.01 to 0.25	0.029	-0.04	-0.27 to 0.19	0.76	0.12	
2 nd tertile	0.12	-0.02 to 0.25	0.09	-0.11	-0.39 to 0.16	0.42	0.14	0.88

3 rd tertile	0.04	-0.09 to 0.16	0.59	-0.14	-0.41 to 0.12	0.29	0.37	
Sodium, mmol/L								
1 st tertile	-0.78	-1.27 to -0.30	0.002	-1.01	-1.70 to -0.32	0.004	0.021	
2 nd tertile	-1.45	-1.89 to -1.00	<0.001	-1.37	-2.09 to -0.64	<0.001	<0.001	0.66
3 rd tertile	-1.21	-1.63 to -0.80	<0.001	-1.17	-1.85 to -0.49	0.001	<0.001	
Potassium, mmol/L								
1 st tertile	0.16	0.09 to 0.24	<0.001	0.24	0.13 to 0.35	<0.001	<0.001	
2 nd tertile	0.20	0.13 to 0.27	<0.001	0.19	0.07 to 0.32	0.002	<0.001	0.19
3 rd tertile	0.30	0.20 to 0.41	<0.001	0.24	0.11 to 0.36	<0.001	<0.001	
eGFR, mL/min/1.73m²								
1 st tertile	-3.00	-4.44 to -1.55	<0.001	-3.56	-6.35 to -0.77	0.012	0.002	
2 nd tertile	-2.98	-4.22 to -1.74	<0.001	-5.06	-7.30 to -2.83	<0.001	<0.001	0.94
3 rd tertile	-3.10	-4.65 to -1.55	<0.001	-4.18	-6.87 to -1.49	0.002	0.002	

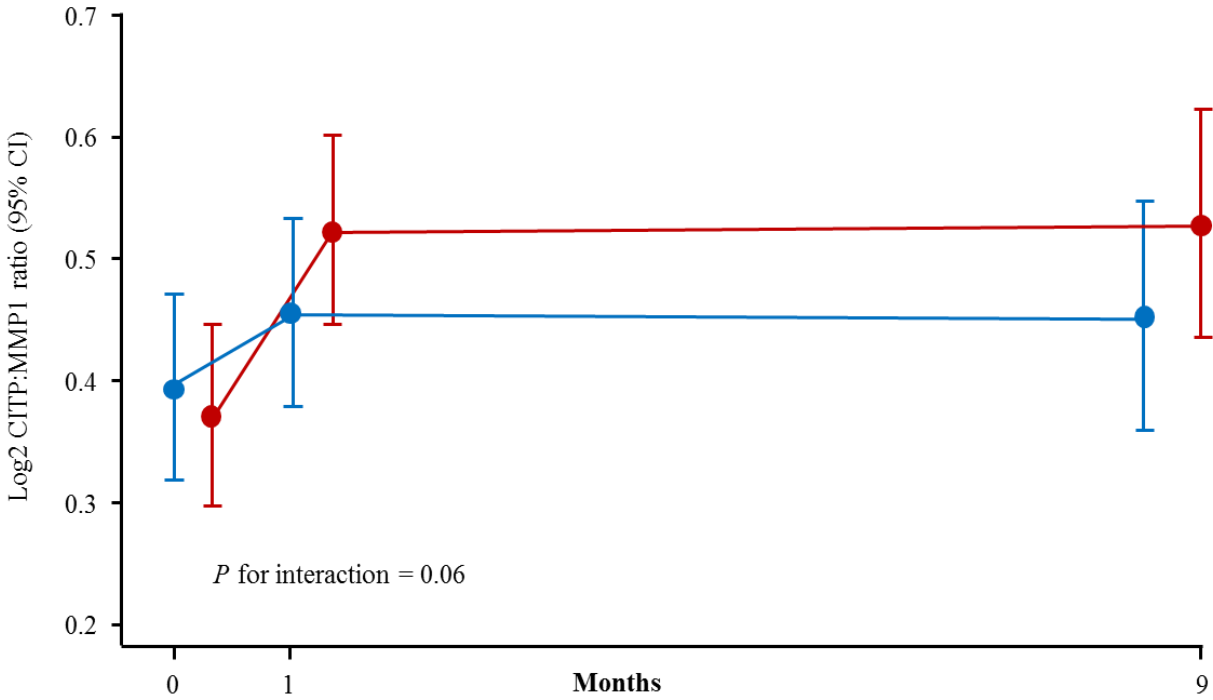
Abbreviations as in table 1. Differences between spironolactone (Spiro) and control (Ctrl) were expressed as mean and 95% confidence interval (CI).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Collagen type-I C-terminal telopeptide to matrix metalloproteinase-1 ratio (CITP:MMP-1) levels (\log_2) according to assigned treatment. 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 (blue line) or with spironolactone (red line) after linear mixed regression analysis with CITP:MMP-1 as the dependent variable, and treatment, visit, their interaction term, and baseline estimated glomerular filtration rate and CITP:MMP-1 values as fixed effects. Mean changes (95% CI) values versus baseline are shown at the bottom.

Supplemental Figure 2. Collagen type-I C-terminal telopeptide to matrix metalloproteinase-1 ratio (CITP:MMP-1) levels (\log_2) according to assigned treatment in patients with low (panel A: 1st tertile), medium (panel B: 2nd tertile) and high (panel C: 3rd tertile) 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 (blue line) or with spironolactone (red line) after linear mixed regression analysis with CITP:MMP-1 as the dependent variable, and treatment, visit, their interaction term, and baseline estimated glomerular filtration rate and CITP:MMP-1 values as fixed effects. Mean changes (95% CI) values versus baseline are shown at the bottom of each panel. * $P < 0.05$ spironolactone vs control, ** $P < 0.01$ spironolactone vs control.

Supplemental Figure 1

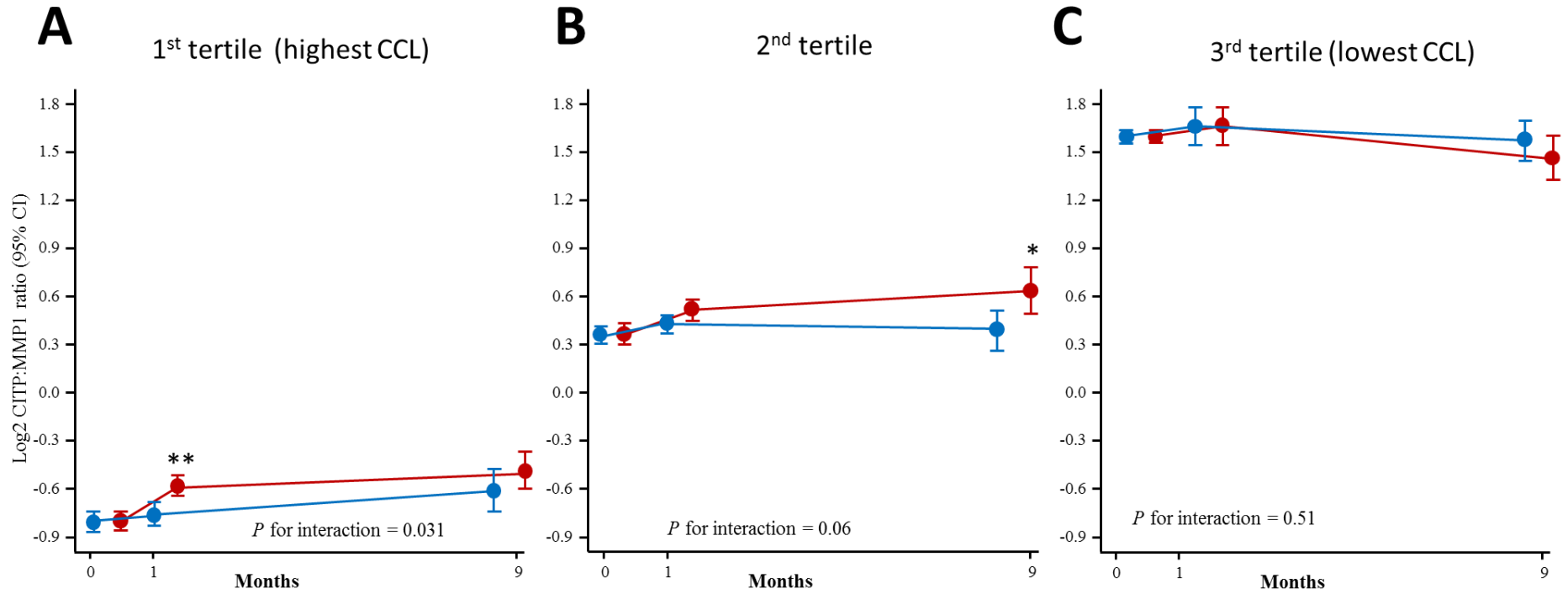


Differences (95% CI) vs month 0

Control	0.06 (0.01 to 0.12)	0.06 (-0.02 to 0.14)
Spironolactone	0.15 (0.09 to 0.21)	0.16 (0.08 to 0.23)



Supplemental Figure 2



Differences (95% CI) vs month 0

Ctrl	0.05 (-0.05 to 0.14)	0.20 (0.06 to 0.33)	0.06 (-0.01 to 0.14)	0.03 (-0.11 to 0.16)	0.06 (-0.04 to 0.17)	-0.03 (-0.15 to 0.10)
Spiro	0.22 (0.13 to 0.30)	0.32 (0.20 to 0.43)	0.15 (0.06 to 0.24)	0.27 (0.12 to 0.43)	0.06 (-0.05 to 0.18)	-0.13 (-0.27 to 0.01)

