Influence of ejection fraction on biomarker expression and response to spironolactone in people at risk of heart failure: findings from the HOMAGE trial

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Abstract

Background: Left ventricular ejection fraction (LVEF) can provide hemodynamic information and may influence the response to spironolactone and other heart failure (HF) therapies.

Aims: To study the patient characteristics and circulating protein associations with LVEF, and whether LVEF influenced the response to spironolactone.

Methods: HOMAGE enrolled patients aged >60 years at high risk of developing HF with a LVEF ≥45%. 527 patients were randomized to either spironolactone or standard-of-care for ≈9 months. 276 circulating proteins were measured using Olink® technology.

Results: 364 patients had available LVEF determined by the Simpson's bi-plane method. The respective LVEF tertiles were: Tertile1:<60% (N=122), Tertile2:60-65% (N=121), and Tertile3:>65% (N=121). Patients with a LVEF>65% had smaller LV chamber size and volumes, and lower natriuretic peptide levels. Compared to patients with a LVEF<60%, those with LVEF>65% had higher levels of circulating c-c motif chemokine ligand-23 and interleukin-8, and lower levels of tissue plasminogen activator, BNP, S100 calcium binding protein A12, and collagen type I alpha 1 chain (COL1A1)**.** Spironolactone significantly reduced the circulating levels of BNP and COL1A1 without significant treatment-by-LVEF heterogeneity: BNP change β=-0.36 Log_2 and COL1A1 change β=-0.16 Log_2 (P<0.0001 for both; interactionP>0.1 for both). Spironolactone increased LVEF from baseline to month 9 by 1.1%, P=0.007.

Conclusion: Patients with higher LVEF had higher circulating levels of chemokines

and inflammatory markers and lower levels of stretch, injury, and fibrosis markers. Spironolactone reduced the circulating levels of natriuretic peptides and type 1 collagen, and increased LVEF.

Key-words: ejection fraction, spironolactone, inflammation, fibrosis.

Introduction

Left ventricular ejection fraction (LVEF) is the ratio of stroke volume to end-diastolic volume of the left ventricle (LV); LVEF can thus provide relevant hemodynamic information, but does not reflect the contractility of the LV.[1]

Over the last two decades, LVEF has been incorporated as an inclusion criterion in heart failure (HF) trials. Patients with HF and reduced EF have been found to benefit markedly from neurohormonal antagonists, whereas HF patients with normal EF have not benefited as markedly as their reduced EF counterparts.[2]

Given the influence of LVEF in the response to HF treatments, studies have explored the relation between patients' characteristics and biomarker expression across the range of LVEF. Some studies suggested that patients with HF who have higher EF have more extra-cardiac comorbidities and higher expression of pathways related to inflammation than patients with lower EF.[3]

The Heart Omics in AGEing (HOMAGE; NCT02556450) trial enrolled people at high risk of developing HF to test the effect of spironolactone (vs. usual care) on circulating markers of fibrosis, natriuretic peptides, blood pressure and cardiac structure and function.[4] Spironolactone reduced the circulating levels of procollagen type-I C-terminal propeptide (PICP) and increased collagen type-I C-terminal telopeptide (CITP), reflecting a decreased in the synthesis and an increase in the degradation of type-I collagen, respectively. In addition, spironolactone reduced blood pressure, NT-pro BNP and left atrial volume, while improving LVEF at 9 months.[5]

In the Aldosterone Antagonist Therapy for Adults With Heart Failure and Preserved Systolic Function (TOPCAT) enrolling patients with HF and a preserved EF (HFpEF), the effect of spironolactone was influenced by LVEF, whereby patients with EF below 55-60% may have benefited from spironolactone.[6] Compared to TOPCAT, HOMAGE enrolled less symptomatic patients with a higher LVEF on average (63% in HOMAGE vs. 56% in TOPCAT).[5, 7]

Given the previously documented differences in patients' characteristics, biomarker expression, and response to spironolactone across LVEF, we aim to study the influence of LVEF on circulating proteins and outcomes in the HOMAGE trial.

Methods

Trial design and population

The HOMAGE trial was a prospective, randomised, open-label, blinded-endpoint (PROBE), multicentre design, in which people at high risk of developing HF were randomly assigned to receive either spironolactone or standard of care/"control" - not receiving spironolactone or other MRA (ClinicalTrials.gov Identifier: NCT02556450). The rationale, trial design and main results have been published.[4, 5]

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 entry criteria included age of 65 or older (amended to 60 years during the course of the trial), cardiovascular risk defined by the presence of coronary artery disease or at least 2 of the following: diabetes mellitus, treated hypertension, microalbuminuria or an abnormal ECG, and a NT-proBNP between 125 and 1,000ng/L or a BNP between 35 and 280ng/L. The main exclusion criteria were

glomerular filtration rate (eGFR) <30 mL/minute/1.73m2, serum potassium >5.0 mmol/L, left ventricular ejection fraction <45%, a diagnosis of HF or treatment with loop diuretics, and atrial fibrillation/flutter.

A total of 527 patients was randomized (265 to spironolactone and 262 to standard of care). The median (percentile$_{25\text{-}75}$) follow-up time was 8.9 (6.0-9.2) months.

Echocardiographic measurements

Echocardiograms were recorded, de-identified and transferred to a core laboratory (University Hospital of Nancy). Blind to treatment allocation, a single experienced echocardiographer (E. B.) measured the echocardiographic variables (including LVEF) 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 mitigate measurement error.

The main LVEF assessment was performed using the Simpson's bi-plane method (N =364). As supplementary analysis, we report the findings from estimates of LVEF calculated from single plane in 4-chamber view images (N =456).

Proteomic biomarkers

Baseline and month 9 (or "last visit") plasma samples were analysed for 276 protein biomarkers by the TATAA-biocenter using the Olink Proseek® Multiplex cardiovascular (CVD) II, CVD III, and inflammation panels. The proteins were determined using high-throughput Olink Proseek® Multiplex 96x96 kits, which measures 92 manually selected proteins simultaneously in 1µl of plasma per kit. Each kit uses a proximity extension assay (PEA) technology with dual-recognition DNA-coupled readout, where 92 oligonucleotide-labelled antibody probe pairs are

allowed to bind to their respective target in the sample. The platform provides Log_2 normalized protein expression (NPX) values with relative quantification. A detailed description of the Olink® technology is depicted on the website: https://www.olink.com/. The abbreviations, full names and respective Olink® multiplex panels of the studied proteins are described in the ***Supplemental Table 1***. In addition, serum PICP was measured using the METRA EIA kit (Quidel Corporation), plasma NT-pro BNP and high sensitivity troponin T (hs-TnT) were assessed by electro-chemiluminescent assays (Roche diagnostics). The assays were performed blinded to treatment allocation.

Statistical analyses

We compared the characteristics of the patients across tertiles of LVEF at baseline using the appropriate tests for continuous and categorical variables. To assess whether the biomarkers were expressed differently between patients with higher (top tertile) and lower (bottom tertile) LVEF, logistic regression analyses were performed comparing the top LVEF tertile (outcome) with the bottom LVEF tertile (referent) with each circulating protein as an independent variable plus age, sex, systolic blood pressure, heart rate, body mass index (BMI) and eGFR as adjustment covariates. To complement the previous step, ordered logistic regression analyses with LVEF tertiles as outcome variable were also performed. To identify the proteins with stronger association with higher (vs. lower) LVEF, a multivariable stepwise forward selection procedure was applied with all the circulating proteins with a P-value <0.05 in the previous step included in the model and the adjustment variables “forced” in the model. A P-value <0.05 was required for a protein to enter and stay in the final model. After selecting the “top” proteins with different expression by LVEF, we have tested whether spironolactone affected the levels of the proteins throughout the

follow-up, using analysis of covariance (ANCOVA) to compare the difference in changes between the control and spironolactone groups. To study whether LVEF could influence the response to spironolactone on the main outcomes of the study, we performed ANCOVA with a treatment-by-LVEF interaction term. The effect of spironolactone on LVEF throughout the follow-up was assess using a mixed effect model with LVEF as dependent variable (measured at baseline, 1 month, and 9 months), treatment (spironolactone vs. control) as independent fixed-effects variable, and age, sex, systolic blood pressure, heart rate, BMI and eGFR as adjustment covariates; the random intercepts were set at the patient "ID" level with an unstructured covariance matrix, meaning that all variances and covariances could vary freely between patients. Statistical analyses were performed using Stata® (version 17, StataCorp LP).

Bioinformatical and network analyses

We used knowledge-based network analysis with induced network approach by consensuspathDB (CPDB) online server (accessed on 25 November 2021) from Max Planck Institute for Molecular Genetics to identify the links among the circulating proteins with different expression according to LVEF tertiles, based on known knowledge of interactions (protein interactions and biochemical interactions).[7] The network analysis also identifies additional proteins limited to the first-degree interactors (intermediate nodes) linking our input proteins (seed nodes), with exclusion of low-confidence interactions and quantified by a z-score ≤20 calculated for each intermediate node. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used to add further nodes to the network. Functional enrichment (GO biological processes) was performed using proteins that were significantly higher or lower in patients with higher vs. lower LVEF at baseline

on a genetic background including only the proteins on the measured OLINK panels to correct for the selected proteins. We only included identified GO-processes when the protein-protein interaction (PPI) enrichment P-value was <0.05.

Results

Patients' characteristics

A total of 364 patients had available baseline LVEF as determined by the Simpson's bi-plane method. The respective LVEF tertiles were: Tertile 1: <60% (N =122), Tertile 2: 60-65% (N =121), and Tertile 3: >65% (N =121). Compared to patients with a LVEF <60% (Tertile 1), those with a LVEF >65% (Tertile 3) had smaller left ventricular end-diastolic diameter (LVEDD) 46.5 vs. 49.4 mm, lower left ventricular end-diastolic and end-systolic volumes indexed to body surface area (LVEDVi and LVESVi) 39.1 vs. 45.6 ml/m^2 and 12.2 vs. 20.7 ml/m^2, respectively, lower NT-pro BNP levels 159.5 vs. 258.0 pg/mL and were more likely to use thiazides 23.1 vs. 12.3%. ***Table 1***. A similar pattern of associations was observed with LVEF tertiles determined from the 4-chamber view only. ***Supplemental Table 2***.

Circulating proteins associated with LVEF

After multivariable stepwise selection with adjustment for clinical variables, compared to patients with a LVEF <60% (Tertile 1), those with a LVEF >65% (Tertile 3) had higher levels of circulating c-c motif chemokine ligand 23 (CCL23; β =+1.79 Log_2 NPX) and interleukin 8 (IL8; β =+0.58 Log_2 NPX), and lower levels of circulating tissue plasminogen activator (TPA; β =-0.83 Log_2 NPX), brain natriuretic peptide (BNP; β =-0.46 Log_2 NPX), S100 calcium binding protein A12 (ENRAGE; β =-0.62 Log_2 NPX) and collagen type I alpha 1 chain (COL1A1; β =-0.92 Log_2 NPX. ***Table 2***. The full list of individual (1-by-1 testing) proteins associated with a LVEF >65% vs.

<60% with adjustment for clinical variables is shown in ***Supplemental Table 3***. Other proteins retained in the multivariable stepwise model included tumor necrosis factor β (TNFβ), CD6, monocyte chemotactic protein 3 (MCP3) and renin (REN), which were higher among patients with LVEF >65% compared to those with LVEF <60%. NT-pro BNP and procollagen type I carboxy-terminal propeptide (PICP) were lower among patients with LVEF >65% compared to those with LVEF <60% (P <0.05 for all). Similar associations were found with ordered logistic regression across LVEF categories (***Supplemental Table 4***), and with LVEF determined from the 4-chamber view only (***Supplemental Tables 5 & 6***). COL1A1 and PICP were well correlated (Rho =0.61, P <0.0001).

There was a significant enrichment of protein-protein interactions among the selected proteins (PPI enrichment p-value =0.0009). A cluster of chemokines was higher in patients with LVEF >65% (*GO:0030593: neutrophil chemotaxis*, FDR 0.00021; CXCL8, CCL23 and CCL7). The circulating chemokines were connected to a lower circulating level of COL1A1 through 3 matrix-metalloproteinases (MMP) which were induced into the network. Even when the network was limited to proteins which remained significant after adjusting for clinical variables, the network showed the same pattern. ***Figure 1***.

Spironolactone effect on top proteins associated with LVEF

Spironolactone reduced the circulating levels of BNP and COL1A1 without significant treatment-by-LVEF heterogeneity: spironolactone vs. control month 9 BNP change β =-0.36 Log_2 NPX and COL1A1 change β =-0.16 Log_2 NPX (P <0.0001 for both; interaction P >0.1 for both). Spironolactone did not significantly change the circulating levels of CCL23, TPA, ENRAGE, and IL8. ***Table 3***.

Spironolactone effect on LVEF

Compared with control, spironolactone increased LVEF in the overall group from baseline to month 9 by 1.1%, P =0.007. The effect of spironolactone on LVEF was more pronounced among patients with LVEF <60% at baseline (Tertile 1) =1.9% and less among patients with LVEF >65% at baseline (Tertile 3) =0.3%, but without significant spironolactone-by-LVEF interaction P =0.24. ***Figure 2***.

The effect of spironolactone on LVEF was not mediated statistically by reductions in BNP or COLA1A1. ***Supplemental Table 7***.

Spironolactone effect on main outcomes of interest by LVEF tertiles

The effect of spironolactone (vs. control) to reduce systolic blood pressure (SBP), PICP, NT-proBNP and left atrial volume indexed to body surface area (LAVi) was not modified by LVEF (interaction P >0.1 for all). ***Table 4***.

Discussion

Our study showed that among patients at risk of developing HF, those with higher LVEF had higher levels of circulating chemokines and inflammatory proteins and lower levels of BNP, collagen type I and proteins related to vascular and endothelial function. Spironolactone reduced the circulating levels of BNP, collagen type I, SBP, and LAVi, irrespective of LVEF, but it did not significantly change the levels of inflammatory proteins. In addition, spironolactone increased LVEF from baseline to month 9, an effect that was more pronounced among patients with lower baseline LVEF. These findings may help better understanding the pathophysiology of patients with preserved EF, particularly those with “supranormal” EF who may have a pro-inflammatory profile with lower expression of fibrosis and myocardial volume overload markers.

Patients with HFpEF and LVEF above 60-65% have been shown to experience an attenuated response to several agents that have been tested in HFpEF, at least regarding HF hospitalizations. The attenuated response at the higher end of LVEF was seen for candesartan in the CHARM-Preserved trial,[8] sacubitril/valsartan in the PARAGON-HF trial,[9] spironolactone in the TOPCAT trial,[6] and, more recently, empagliflozin in the EMPEROR-Preserved trial;[10] still, in EMPEROR-Preserved the attenuation of effect with empagliflozin seemed to have occurred only in patients with EF of 65% or greater.[11] The mechanisms by which patients at the higher end of the EF spectrum do not respond similarly to patients with lower EFs, using the same agents, are not well-established. Some studies have suggested that patients with higher EFs constitute a different phenotype with high ventricular-arterial stiffening with aging and hypertension as contributing factors (e.g., in HOMAGE the higher the LVEF the more frequent was the use of thiazide-type diuretics).[12] Such patients have smaller LV diameter and lower systolic and diastolic volumes; thus, the LV end-diastolic pressures may be lower.[13] Mechanistic studies have shown that patients with higher LVEF have a pro-inflammatory profile,[14] with lower expression of cardiac stretch and injury markers.[3, 15]

Patients participating in HOMAGE did not have overt HF signs and symptoms, but did have high natriuretic peptides and alterations of cardiac structure and function.[5] To a great extent, the present study replicates previous findings in HFpEF and expands the phenotyping of patients with “normal and supranormal” EF. In HOMAGE, compared to patients in the lower LVEF tertile (<60%), those in the upper tertile of LVEF (i.e., >65%) had smaller LV with lower systolic and diastolic volumes and natriuretic peptide levels, suggesting that these patients have lower LV end-diastolic pressures. The expression of chemokines and pro-inflammatory markers

(CCL23 and IL8) was also higher among patients in the upper LVEF tertile. CCL23 is a chemokine serving as chemotactic factor for monocytes/macrophages, dendritic cells and lymphocytes, which may play a role both as a circulating and tissue inflammatory molecule, up-regulating the release of pro-inflammatory cytokines such as TNFα.[16] IL8 is a major mediator of inflammatory response, involved in neutrophil chemotaxis, angiogenesis, atherogenesis, and cancer.[17] In patients with chronic HF, IL8 was independently associated with poor outcomes.[18] In HOMAGE, both the CCL23 and IL8 levels were not significantly modified by spironolactone.

Patients in the upper LVEF tertiles expressed lower circulating levels of BNP and collagen type I, which is also in concordance with prior HF studies showing that patients with higher EF had lower levels of cardiac stretch and injury markers.[3, 15, 19] In HOMAGE, spironolactone significantly reduced collagen type I-related biomarkers (both COL1A1 and PICP) and natriuretic peptides (both BNP and NT-pro BNP),[5, 20] irrespective of LVEF. The effect of spironolactone to reduce SBP and LAVi was also not influenced by baseline LVEF.

Beyond the lower levels of BNP and collagen type I, patients in the upper LVEF tertile also expressed lower circulating levels of TPA and ENRAGE. TPA is produced by vascular endothelial cells and activates clot dissolution in the presence of fibrin by converting plasminogen to plasmin.[21] Higher TPA levels have been associated with higher risk of cardiovascular events.[22] ENRAGE is involved in calcium-dependent signal transduction pathways and may act in the regulation of cytoskeletal components.[23] Higher ENRAGE levels have been associated with poor cardiovascular and HF outcomes.[24] In HOMAGE, both the TPA and ENRAGE levels were not significantly changed with spironolactone treatment.

LVEF was significantly increased with spironolactone treatment from baseline to month 9 (LVEF change =1.1%), despite the absence of a significant interaction, the effect of spironolactone to improve LVEF was more pronounced among patients in the lower LVEF tertile (LVEF <60%), who had more margin for improvement. Spironolactone has been shown to improve systolic function, determined by LV longitudinal strain, in the TOPCAT trial.[25] However, in a subset of 239 patients enrolled in TOPCAT, LVEF was not significantly improved with spironolactone treatment during 12 to 18 months (LVEF change: +0.6%, P =0.33).[26] In the ALDO-DHF trial, spironolactone improved LVEF at 12 months by +1.6% (P =0.04).[27] These findings, together with HOMAGE, suggest that spironolactone may have, at least, a modest effect to improve LVEF in patients with preserved ejection fraction.

Limitations

Despite the external replication of our main findings, these results should be regarded as hypothesis-generating given the post-hoc nature of our study, the relatively small sample size of LVEF tertiles, and the lack of mechanism confirmation at a cellular level. As per inclusion criteria, HOMAGE included only asymptomatic patients with a LVEF of 45% or greater and these findings cannot be generalized to symptomatic patients or those with lower ejection fractions; still, tertile 1 (LVEF <60%) include patients with “mildly reduced” or “mid-range” LVEF who may present a phenotype similar to patients with reduced EF.[28] We did not find treatment effect modification by LVEF categories (regarding collagen markers, natriuretic peptides and blood pressure); however, HOMAGE was a mechanistic trial to evaluate the impact of spironolactone on circulating collagen markers in a low-risk population who did not experience HF hospitalizations or fatal events. Therefore, the findings of TOPCAT could not be replicated herein.

Conclusions

In patients at risk of developing HF enrolled in the HOMAGE trial, those with higher LVEF had higher levels of circulating inflammatory markers and lower levels of stretch, injury, and fibrosis markers. These finding support a different phenotype of patients with “supranormal” EF, which may help explaining why such patients may not respond to HF therapies.

Ethics approval and consent to participate

The study was approved by all relevant ethics committees and regulatory bodies. All participants provided written informed consent prior to study specific procedures.

Consent for publication

There is no data of individual persons included in the manuscript.

Competing interests

The authors have no relevant conflicts of interest to disclose with regards to the content of this manuscript.

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Table 1. Baseline patients' characteristics by tertiles of LVEF

Characteristic	LVEF tertiles			P-value
	<60%	60-65%	>65%	
N.	122	121	121	
Age, years	72.1 (68.3, 77.4)	73.4 (69.3, 78.7)	71.5 (68.2, 76.9)	0.12
Men, n (%)	94 (77.0%)	95 (78.5%)	84 (69.4%)	0.21
CAD, n. (%)	95 (77.9%)	90 (74.4%)	85 (70.2%)	0.40
Hypertension, n. (%)	88 (72.1%)	90 (74.4%)	98 (81.0%)	0.25
Diabetes, n. (%)	48 (39.3%)	52 (43.0%)	53 (43.8%)	0.76
BMI, Kg/m^2	27.4 (25.0, 30.2)	28.2 (25.1, 31.6)	28.3 (25.3, 31.8)	0.20
Waist circ., cm	100.0 (93.0, 108.0)	101.5 (95.0, 109.0)	101.5 (95.0, 111.0)	0.30
SBP, mmHg	140.0 (128.0, 152.0)	141.0 (130.0, 156.0)	142.0 (129.0, 159.0)	0.23
DBP, mmHg	79.0 (73.0, 85.0)	79.0 (72.0, 84.0)	77.0 (70.0, 84.0)	0.17
Heart rate, bpm	62.0 (56.0, 69.0)	58.0 (54.0, 65.0)	59.0 (54.0, 66.0)	0.022
LVEF, % *	55.0 (52.3, 58.1)	62.9 (61.6, 64.0)	68.5 (66.4, 71.5)	<0.001
LVMi, g/m^2	100.6 (87.6, 115.5)	93.8 (81.1, 111.8)	88.9 (77.8, 101.6)	0.001
LAVi, ml/m^2	31.2 (27.3, 37.2)	30.9 (24.3, 37.7)	30.6 (25.9, 35.6)	0.35
E/e'	9.1 (7.3, 11.9)	9.4 (7.9, 11.1)	9.5 (8.0, 11.6)	0.75
E/A ratio	0.8 (0.6, 1.0)	0.9 (0.7, 1.0)	0.9 (0.7, 1.0)	0.004
LVEDD, mm	49.4 (46.1, 53.9)	47.4 (44.2, 50.3)	46.5 (44.2, 50.2)	<0.001
LVEDV, ml/m^2	45.6 (38.3, 54.5)	41.7 (37.4, 48.5)	39.1 (33.3, 45.7)	<0.001
LVESV, ml/m^2	20.7 (16.8, 24.9)	15.6 (13.3, 18.0)	12.2 (9.8, 14.5)	<0.001
eGFR, ml/min/1.73m^2	75.5 (64.2, 85.3)	70.5 (60.9, 82.9)	76.1 (66.5, 88.1)	0.061
eGFR <60, n. (%)	21 (17.2%)	27 (22.3%)	22 (18.2%)	0.56
Urea, mmol/L	8.6 (5.7, 13.6)	10.0 (6.1, 15.0)	8.5 (5.8, 13.6)	0.13
Hemoglobin, g/dl	14.0 (13.1, 14.9)	14.3 (13.5, 15.2)	13.8 (13.0, 14.7)	0.064
Sodium, mmol/L	140.0 (138.0, 141.0)	140.0 (138.0, 141.0)	139.0 (137.0, 141.0)	0.13
Potassium, mmol/L	4.4 (4.1, 4.6)	4.3 (4.1, 4.6)	4.3 (4.1, 4.5)	0.36
NT-pro BNP, pg/mL	258.0 (153.4, 451.9)	194.0 (121.4, 298.8)	159.5 (111.8, 288.3)	<0.001
Anti-platelet, n. (%)	96 (78.7%)	97 (80.2%)	98 (81.0%)	0.90
Beta-blocker, n. (%)	86 (70.5%)	83 (68.6%)	85 (70.2%)	0.94
ACEi/ARB, n. (%)	91 (74.6%)	94 (77.7%)	93 (76.9%)	0.84
CCB, n. (%)	23 (18.9%)	22 (18.2%)	24 (19.8%)	0.95
Thiazide, n. (%)	15 (12.3%)	16 (13.2%)	28 (23.1%)	0.040
Statin, n. (%)	104 (85.2%)	99 (81.8%)	103 (85.1%)	0.71

Legend: CAD, coronary artery disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEF, left ventricular ejection fraction; LVM, left ventricular mass indexed to body surface area; LAV, left atrial volume indexed to body surface area; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; eGFR, estimated glomerular filtration rate; ACEi/ARB, angiotensin converting enzyme/angiotensin receptor blocker; CCB, calcium channel blocker; *LVEF analyzed by the Simpson bi-plane method.

Table 2. Top proteins associated with LVEF

Protein (Log_2 NPX)	**Coefficient (95%CI) LVEF: >65% vs. <60%**	**P-value**
CCL23	+1.79 (+0.95 to +2.63)	<0.0001
TPA	-0.83 (-1.27 to -0.39)	<0.0001
BNP	-0.46 (-0.75 to -0.18)	0.002
ENRAGE	-0.62 (-1.05 to -0.18)	0.005
COL1A1	-0.92 (-1.67 to -0.18)	0.015
IL8	+0.58 (+0.09 to +1.07)	0.019

Legend: CCL23, c-c motif chemokine ligand 23; TPA, tissue plasminogen activator; BNP, brain natriuretic peptide; ENRAGE, S100 calcium binding protein A12; COL1A1, collagen type I alpha 1 chain; IL8, interleukin 8.
LVEF obtained with Simpson bi-plane method.
Multivariable stepwise forward logistic regression model with age, sex, systolic blood pressure, heart rate, body mass index, and eGFR "forced" into the model, and all circulating proteins with a P-value of <0.05 in the 1-by-1 analysis entered in the model (BNP, NT-pro BNP, CCL23, COL1A1, TPA, TNFB, PICP, IL8, CD6, MCP3, ENRAGE, REN; see Supplemental Table 3).

Table 3. Effect of spironolactone on the top proteins associated with LVEF

Protein (Log_2 NPX)	**Month 1**	**Month 9**	**Treatment-by-LVEF interaction P**
CCL23	-0.04 (-0.11 to +0.02) P =0.21	+0.01 (-0.06 to +0.08) P =0.72	0.72
TPA	+0.03 (-0.15 to +0.21) P =0.74	+0.11 (-0.07 to +0.29) P =0.24	0.20
BNP	-0.45 (-0.61 to -0.29) P <0.0001	-0.36 (-0.52 to -0.19) P <0.0001	0.60
ENRAGE	+0.03 (-0.08 to +0.15) P =0.57	-0.05 (-0.16 to +0.06) P =0.39	0.60
COL1A1	-0.09 (-0.15 to -0.03) P =0.005	-0.16 (-0.23 to -0.10) P <0.0001	0.89
IL8	-0.01 (-0.12 to +0.09) P =0.81	-0.09 (-0.20 to +0.01) P =0.078	0.67

Legend: CCL23, c-c motif chemokine ligand 23; TPA, tissue plasminogen activator; BNP, brain natriuretic peptide; ENRAGE, S100 calcium binding protein A12; COL1A1, collagen type I alpha 1 chain; IL8, interleukin 8.
LVEF obtained with Simpson bi-plane method.
Caption: BNP and COL1A1 were decreased with spironolactone over time, without effect modification by LVEF.

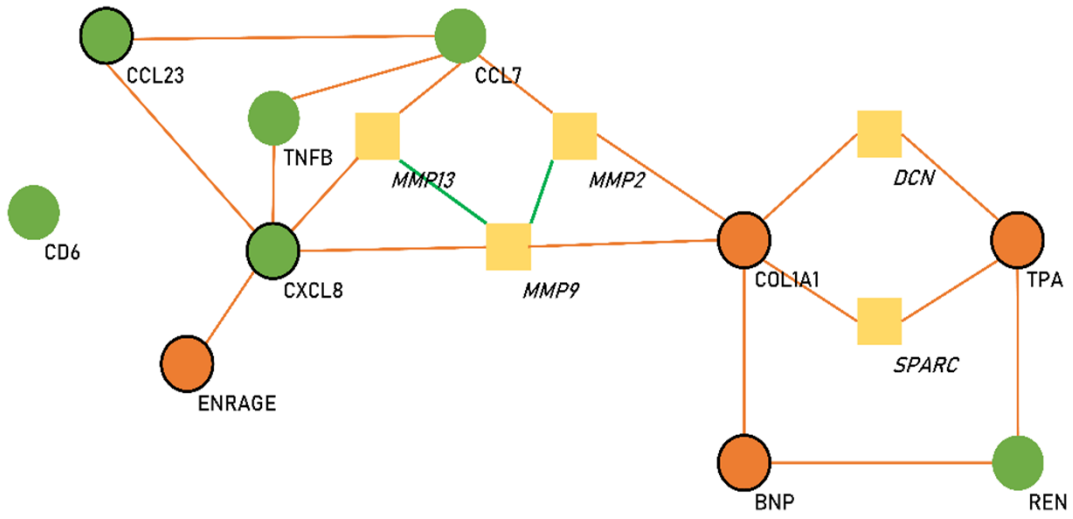
Table 4. Effect of spironolactone on main outcomes by LVEF tertiles

Outcome/LVEF tertile	Coefficient (95%CI)	Treatment-by-LVEF interaction P
SBP change (mmHg)		
LVEF <60%	-7.3 (-13.1 to -1.5)	0.48
LVEF 60-65%	-12.1 (-17.7 to -6.6)	
LVEF >65%	-10.6 (-16.3 to -4.9)	
PICP change (µg/l)		
LVEF <60%	-12.4 (-20.0 to -5.0)	0.54
LVEF 60-65%	-7.6 (-15.2 to 0.0)	
LVEF >65%	-7.0 (-14.4 to +0.4)	
NT-pro BNP change (pg/ml)		
LVEF <60%	-78 (-194 to +39)	0.90
LVEF 60-65%	-99 (-215 to +18)	
LVEF >65%	-61 (-177 to +55)	
LAVi change (ml/m^2)		
LVEF <60%	-2.8 (-5.1 to -0.4)	0.76
LVEF 60-65%	-1.5 (-3.7 to +0.6)	
LVEF >65%	-2.0 (-4.3 to +0.3)	

Legend: SBP, systolic blood pressure; PICP, procollagen type I carboxy-terminal propeptide; LAVi, left atrial volume indexed to body surface area.
Change from baseline to month 9.

Figure 1. Network analysis relating the top proteins associated with LVEF

Legend: CCL23, c-c motif chemokine ligand 23; TPA, tissue plasminogen activator; BNP, brain natriuretic peptide; ENRAGE, S100 calcium binding protein A12; COL1A1, collagen type I alpha 1 chain; IL8, interleukin 8; REN, renin; MMP, matrix metalloproteinase; SPARC, secreted protein acidic and cysteine rich; DCN, decorin.

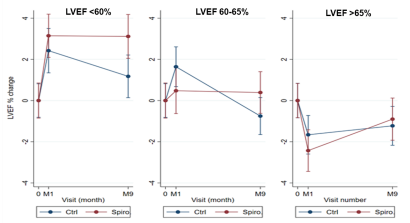


LEGEND

- Increased
- Decreased
- Intermediate node
- Protein interaction
- Biochemical interaction
- Significant after multivariable analysis
- Not significant after multivariable analysis

Figure 2. Spironolactone effect on LVEF by tertiles of LVEF

Legend: Ctrl, control; Spiro., spironolactone; 0, baseline; M1, month 1; M9, month 9.
Spironolactone vs. Control effect on LVEF:
Tertile 1 LVEF <60%:
M1 =+0.7 (-0.8 to +2.2) %, P =0.34; M9 =+1.9 (+0.5 to +3.4) %, P =0.011.
Tertile 2 LVEF 60-65%:
M1 =-1.2 (-2.6 to +0.3) %, P =0.12; M9 =+1.1 (-0.2 to +2.5) %, P =0.098.
Tertile 3 LVEF >65%:
M1 =-0.8 (-2.1 to +0.6) %, P =0.27; M9 =+0.3 (-1.1 to +1.7) %, P =0.64.
Spironolactone-by-LVEF interaction P =0.24.
Overall effect:
M1 =-0.4 (-1.3 to +0.4) %, P =0.31; M9 =+1.1 (+ 0.3 to +1.9) %, P =0.007.
Overall joint P-value =0.012.
Caption: Spironolactone improved LVEF from baseline to month 9.


LVEF <60%
LVEF 60-65%
LVEF >65%
LVEF % change
-4
-2
0
2
4
0 M1
M9
Visit (month)
Visit number
Ctrl
Spiro