The association between markers of type I collagen synthesis and echocardiographic response to spironolactone in patients at risk of heart failure: findings from the HOMAGE trial

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Abstract

Background

Procollagen type I C-terminal propeptide (PICP) and procollagen type III N-terminal propeptide (PIIINP) are markers reflecting collagen synthesis in cardiac fibrosis. However, they may be influenced by the presence of noncardiac comorbidities (*e.g.*, ageing, obesity, renal impairment). Understanding the associations between markers of collagen synthesis and abnormalities of cardiac structure and function is important to screen for myocardial fibrosis and monitor the antifibrotic effect of medications.

Methods

The HOMAGE (Heart OMics in Aging) trial showed that spironolactone decreased serum PICP concentrations and improved cardiac remodeling over 9 months in a population at risk of developing heart failure (HF). We evaluated the associations between echocardiographic variables, PICP, PIIINP and galectin-3 at baseline and during the course of the trial.

Results

Among 527 individuals (74±7years, 26% women), median serum concentrations of PICP, PIIINP and galectin-3 were 80.6µg/L (65.1-97.0), 3.9µg/L (3.1-5.0) and 16.1µg/L (13.5-19.7), respectively. After adjustment for potential confounders, higher serum PICP was significantly associated with left ventricular hypertrophy, left atrial enlargement, and greater ventricular stiffness (all p-values<0.05), whereas serum PIIINP and galectin-3 were not (all p-values>0.05). In patients treated with spironolactone, a reduction in serum PICP during the trial was associated with a decrease in E/e′ (adjusted-beta [95% CI] =0.93 [0.14-1.73]; p=0.022).

Conclusions

In individuals at high risk of developing HF, serum PICP was associated with cardiac structural and functional abnormalities, and a decrease in PICP with spironolactone was correlated with improved diastolic dysfunction as assessed by E/e′. In contrast, no such associations were present for serum PIIINP and galectin-3.

Keywords: collagen markers; myocardial fibrosis; spironolactone; diastolic function; cardiac structural remodeling; heart failure.

Introduction

Myocardial fibrosis is often accompanied by increased left ventricular (LV) stiffness, hypertrophy, and diastolic functional abnormalities, all of which may contribute to the development of heart failure (HF) [1, 2]. Serum concentrations of procollagen type I C-terminal propeptide (PICP) and procollagen type III N-terminal propeptide (PIIINP) are surrogate markers of systemic and, potentially, myocardial fibrosis generated during the extracellular conversion of procollagen to mature fibril-forming collagen type I and III, respectively [3]. In addition, serum concentration of galectin-3, a mediator of aldosterone-induced cardiac fibrosis [4], correlates with serum markers associated with extracellular matrix turnover [5].

Whether these markers specifically reflect alterations in cardiac structure and function remains insufficiently established [6]. This is clinically important, as "cardiac-specific" markers could be useful for screening cardiac fibrosis and identifying populations at high risk of developing HF [7]. Markers of collagen synthesis are commonly used to quantify the severity of myocardial fibrosis in various settings [8, 9], and predict therapeutic response to cardiac remodeling and cardiovascular events in HF with a preserved ejection fraction (HFpEF) [10, 11]. Several non-cardiac comorbidities promote fibrosis [12, 13], and thus serum PICP and PIIINP may be potentially influenced by ongoing collagen deposition in many extra-cardiac tissues (*e.g.*, skin, lung, liver, kidney, heart) [7, 14].

In the Heart "OMics" in AGEing (HOMAGE) trial, spironolactone reduced serum PICP and improved cardiac remodeling in people with preclinical HF [15]. In the present analysis, we (i) explore whether clinical profiles influence serum PICP, PIIINP and galectin-3, (ii) assess the associations between the collagen-related markers, abnormal echocardiographic structure and function, and (iii) assess the associations between changes in collagen-related markers and changes in echocardiographic variables as a consequence of spironolactone treatment.

Methods

Study population

The HOMAGE trial has been described previously [16]. Patients aged ≥65 years (amended to ≥60 years during the course of the trial) with established coronary artery disease or at least two criteria indicating cardiovascular disease (*i.e.* hypertension, diabetes mellitus, microalbuminuria or an abnormal electrocardiogram), and those with a plasma amino-terminal pro-B-type natriuretic peptide (NT-proBNP) of 125-1,000 pg/mL or B-type natriuretic peptide 35-280 pg/mL were included. Patients with a pre-existing diagnosis of HF, a left ventricular ejection fraction (LVEF)<45%, atrial fibrillation, an estimated glomerular filtration rate (eGFR) [17] <30 mL/min/1.73m^2 or a serum potassium >5.0 mmol/L were excluded. The trial was approved by relevant ethics committees and regulatory bodies. Participants provided written informed consent (ClinicalTrials.Gov Identifier: NCT02556450).

Laboratory assays

Serum PICP was measured by enzyme immunoassay (METRA; Quidel Corporation®), PIIINP by radio-immunoassay (Orion Diagnostica®), and galectin-3 by enzyme-linked immunosorbent assay (ELISA), blinded to clinical and echocardiographic data, as previously reported [15].

Echocardiogram

Echocardiograms were recorded, de-identified and transferred to the Nancy University Hospital Clinical Investigation Center. LV volumes and LVEF were calculated using the biplane method of disks summation (modified Simpson's rule), while left atrial (LA) volume was assessed using the biplane area-length method from apical views. Diastolic function was assessed from the pattern of mitral inflow by pulsed-wave Doppler. Mitral annular early diastolic velocity (e′) was assessed at the septal and lateral sites of the mitral annulus using tissue Doppler imaging. E/A ratio, average e′ and

average E/e′ ratio were calculated [18]. Tricuspid annular plane systolic excursion (TAPSE) measurement was obtained as recommended [19].

Baseline measurements were carried out by a single experienced echocardiographer (E.B.), blinded to clinical data, using dedicated software (Echo PAC, GE Healthcare). Measurements were repeated at least two months later, blinded 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. Our center's measurement reproducibility by experienced echocardiographers has been previously reported [20].

Dilated LV end-diastolic volume (LVEDV) was defined by an indexed LVEDV (LVEDVi) >74ml/m² in men or >61mL/m² in women, decreased LVEF by LVEF <52% in men or LVEF <54% in women, LV hypertrophy by indexed LV mass (LVMi) >115g/m² in men or >95g/m² in women, LA volume enlargement by indexed LA volume (LAVi) >34 mL/m², and abnormal e′ by a septal e′ <7cm/sec or lateral e′ <10cm/sec [18, 21].

Statistical analysis

Categorical variables are presented as frequencies (percentages) and continuous variables as mean ± standard deviation or median (25th and 75th percentiles) depending on distribution. Comparisons of baseline characteristics according to tertiles of collagen-related markers were analyzed using ANOVA, Kruskal-Wallis and χ^2 tests, as appropriate. Unadjusted correlations are presented using Spearman's correlation coefficients. Patients were categorized by tertiles of baseline PICP (1st tertile: <71.9µg/L, 2nd tertile: 71.9-91.2µg/L, 3rd tertile: >91.2µg/L), PIIINP (1st tertile: <3.4µg/L, 2nd tertile: 3.4-4.6µg/L, 3rd tertile: >4.6µg/L), and galectin-3 (1st tertile: <14.5µg/L, 2nd tertile: 14.5-18.3µg/L, 3rd tertile: >18.3µg/L).

Multinominal logistic regression analyses with backward selection were tested to explore baseline clinical factors associated with tertiles of each collagen-related marker (with the lowest tertile group as a reference). Candidate variables were as follows: age, sex, body mass index (BMI), ratio of waist and hip circumference, exercise performance (as number of shuttles completed in a shuttle walk test), medical history (hypertension, diabetes, coronary artery disease, previous myocardial infarction, a prior percutaneous coronary intervention, coronary artery bypass graft, stroke/transient ischemic attack [TIA], chronic obstructive pulmonary disease and implanted cardiac device), visual analogue scale (on which scores range from 0 to 100, with higher scores indicating dyspnea with milder activities), systolic blood pressure (BP), diastolic BP, heart rate, QRS duration in electrocardiogram, and laboratory findings (hemoglobin, sodium, potassium, and eGFR). These variables had a small proportion of missing values (<10%), and no imputation was performed. Subsequently, logistic regression analyses were performed to study the association between collagen-related markers, and abnormalities of cardiac structure and function after adjustment for clinical confounders selected from the models above. Linear regression analyses, in addition, were used to assess the associations between changes in collagen-related markers and changes in echocardiographic variables during the follow-up period separately in patients assigned to spironolactone and placebo. The models were adjusted for clinical confounders selected as above plus baseline collagen-related markers and echocardiographic variables.

Statistical analyses were performed using R version 3.4.0 (R Development Core Team, Vienna, Austria). A two-sided p-value <0.05 was considered statistically significant.

Results

Baseline characteristics, and clinical factors associated with collagen synthesis markers

Among the 527 participants included in the study, mean age was 74±7 years, 26% were women, one-third (34 %) had a BMI ≥30kg/m², 78% had hypertension, and median NT-proBNP was 214 pg/mL

(25th and 75th centiles 137-356 pg/mL). The mean LVEF was 62 ±7%, 29% had LV hypertrophy, 34% had LA enlargement and 87% had decreased e′.

Median serum concentrations of PICP, PIIINP and galectin-3 were 80.6 µg/L (65.1-97.0), 3.9 µg/L (3.1-5.0) and 16.1 µg/L (13.5-19.7), respectively (*Supplemental Figure 1)*, and the correlations between them were weak (Spearman's rho<0.20) (*Supplemental Table 1)*. The baseline characteristics of the patients divided by tertiles of serum PICP and PIIINP are shown in *Table 1*. Patients with higher serum PICP were more likely to be women, were more likely to have a history of stroke or TIA, had lower eGFR, and greater LVMi and LAVi. Those with higher serum PIIINP were older, had a higher BMI and waist circumference, were more likely to have a history of hypertension, had lower eGFR and greater LVMi. In addition, those with higher serum galectin-3 were older, were more likely to have a history of hypertension and diabetes, had lower eGFR, greater LVMi, higher E/e′ ratio and higher serum concentrations of collagen type-1 C-terminal telopeptide (CITP). *Supplemental Table 2*.

Higher BMI and lower eGFR were associated with higher serum PICP, whereas older age and higher BMI were associated with higher serum PIIINP. Older age, a history of diabetes and lower eGFR were associated with higher serum galectin-3 (*Table 2).*

Associations between collagen synthesis markers and echocardiographic variables

In a multinomial logistic model for serum PICP with the lowest tertile as a reference, those in the upper tertile group were more likely to have LV hypertrophy (adjusted-OR [95%CI] =1.89 [1.08-3.32]; p=0.026), LA enlargement (adjusted-OR=2.01 [1.21-3.37]; p=0.007) and abnormal e′ (adjusted-OR =2.32 [1.12-5.06]; p=0.028). By contrast, serum PIIINP and galectin-3 were not significantly associated with echocardiographic structural or functional abnormalities (all p-value>0.05) (*Table 3*).

Similar associations were observed regardless of the type of LV mass and LA volume variables considered (i.e., absolute, indexed to body surface area, and height). *Supplemental Table 3*.

Associations between changes in collagen markers and changes in echocardiographic variables

After adjustment for clinical confounders (selected from the results of Table 2), in subjects treated with spironolactone, a decrease in serum PICP during follow-up was significantly associated with a decrease in E/e′ (adjusted-beta [95% CI] =1.02 [0.21-1.83]; p=0.014) but not in those not treated with spironolactone; however, the interaction test was not significant (p>0.10). An increase in serum galectin-3 from baseline to the final visit was significantly associated with a decrease in LAVi in people treated with spironolactone treatment (adjusted-beta [95% CI] =-5.69 [-8.58 - -2.79], p<0.001) but not in those not treated with spironolactone (p for interaction=0.005). No other significant associations between changes in collagen-related markers and changes in echocardiographic variables were observed (*Table 4*).

Discussion

In a population at a high risk of developing HF, we found that age and/or comorbidities (particularly obesity and lower eGFR) were associated with increasing concentrations of circulating markers of collagen synthesis; however, independent of these confounders, higher serum PICP was associated with abnormalities of diastolic function (specifically, with greater LA volume indexed for body surface area and lower e′). In contrast, serum PIIINP and galectin-3 were not associated with abnormalities in any of the measures of cardiac structure or function. In addition, in individuals treated with spironolactone, a decrease in serum PICP during follow-up paralleled a decrease in E/e′. These findings suggest that PICP might be useful in screening individuals for abnormalities in cardiac structure and function associated with cardiac fibrotic remodeling, and that changes in PICP might be used to monitor any subsequent change in diastolic function due to the antifibrotic effect of spironolactone [22].

In the current analysis of the HOMAGE trial including people with preclinical HF, values of PICP and PIIINP were comparable to those in patients with resistant hypertension [23] and lower than those in patients with HFpEF [24, 25]. Higher BMI was associated with higher serum PICP and PIIINP, which is consistent with previous reports [8, 26]. We also observed that lower eGFR was associated with higher serum PIIINP. This finding is supported by evidence suggesting that pathophysiological mechanisms in chronic kidney disease such as hemodynamic overload, biochemical mediators (for example, angiotensin II and oxidative stress) and inflammation may alter fibroblasts and collagen synthesis as measured by serum PIIINP in the heart or in the kidney [27-29].

Consistent with prior reports, we found that serum galectin-3 correlated with echocardiographic measures of diastolic function and serum CITP, a marker of collagen degradation [5, 30]. However, after adjustment for clinical confounders (*i.e.*, old age, diabetes and renal function) as previously reported [31, 32], serum galectin-3 was no longer significantly associated with cardiac structure and function. Interestingly, an increase in serum galectin-3 was associated with a reduction in LAVi in subjects assigned to spironolactone. Galectin-3 is increased directly by aldosterone via mineralocorticoid receptor antagonism [33], and in the main results of the HOMAGE study, spironolactone caused a significant increase in serum galectin-3 , perhaps as a consequence of increases in aldosterone due to mineralocorticoid receptor blockade by spironolactone [15]. Our observations might be explained by a simultaneous fall in LV filling pressures (as reflected in the LAVi) and rise in serum galectin-3, as co-variates, caused by mineralocorticoid receptor antagonism with spironolactone [33].

Some evidence suggests that both collagen synthesis markers, PICP and PIIINP, are associated with myocardial fibrosis confirmed by endomyocardial biopsies [34-36]. However, PICP originates directly from the synthesis of collagen type I, whereas PIIINP originates from partially processed procollagen molecules on the surface of collagen type III fibers [3]. Net release from the

heart into the circulation has been reported only for PICP [34]. The myocardial collagen matrix consists primarily of type I collagen, which is composed of large-diameter fibres [37]. Collectively, these mechanisms may explain our observations that high serum PICP, but not PIIINP, is associated with LV hypertrophy, stiffness, and LA enlargement. Interestingly, we found that a decrease in PICP with spironolactone treatment was significantly and linearly associated with improvements in diastolic function as assessed by changes in E/e′. PICP may also play a key role in the assembly of collagen type I molecules into collagen type I fibrils [38]. The reduction of serum PICP by spironolactone, therefore, may have double anti-fibrotic beneficial effects: reduced availability of mature collagen type I molecules and reduced assembly of these molecules to form collagen type I fibrils. These data support the hypothesis that serum PICP (rather than serum PIIINP and galectin-3) may be related to abnormalities of cardiac structure and function, and could be used to monitor the anti-fibrotic action of spironolactone involved in its reverse remodeling effect. In support of this possibility, falls in serum PICP with treatment are associated with reduction of fibrosis in endomyocardial biopsies both in non-HF [39] and HF patients [40].

Of interest, the impact of myocardial fibrosis is determined by the quantity of collagen deposition (captured by PICP), as well as by the degree of collagen cross-linking, which increases the stiffness and resistance to degradation of collagen fibers. Indeed, previous sub-studies in patients with HFpEF from the Aldosterone Receptor Blockade in Diastolic Heart Failure (ALDO-DHF) trial[25] and from the HOMAGE trial[41] showed that baseline collagen type I cross-linking (non-invasively estimated by the ratio of CITP to matrix metalloproteinase [MMP-1]) influenced the beneficial cardioprotective effects of spironolactone, with patients with low collagen cross-linking showing the highest improvement in parameters associated with diastolic dysfunction and remodelling (e.g. E/e′ in ALDO-DHF and LAVi in HOMAGE). Therefore, we cannot discard that the associations reported in the current study between PICP and structural and functional parameters might be influenced by the degree of collagen cross-linking.

Our findings in preclinical HF were not in line with a prior report showing that spironolactone treatment reduced serum PIIINP in patients with severe HF and a reduced LVEF [42]. The different etiology of HF may partly influence the relevance of collagen type I and type III [43]. Therefore, the substantial differences in population characteristics, comorbidity burden, LVEF and severity of HF are likely responsible for differences in the response to spironolactone treatment between these studies [44]. The present study should be regarded as hypothesis generating and larger sample sized studies should be warranted to validate the finding that PICP is more specific for myocardial fibrosis than PIIINP and galectin-3 in other clinical settings.

Our study has several limitations. This is a post-hoc analysis of the HOMAGE trial, hence has the limitations inherent in exploratory analyses. Despite different patterns observed between spironolactone treatment groups, the interaction between changes in serum PICP and changes in E/e′ with regards to spironolactone use was not significant; however, the moderate sample size of our population may limit the statistical power of such interaction test [45]. Of note, the interaction was significant when considering galectin-3 and LAVi, which sizably strengthen our pathophysiological perspective. There were no data regarding global longitudinal strain, which better captures subtle systolic impairments; however this echocardiographic variable requires more sophisticated equipment as well as professional skill which can limit its availability [46].

Conclusions

In individuals at a high risk of developing HF, serum PICP was associated with abnormalities of cardiac structure and function, and a decrease in serum PICP with spironolactone treatment was associated with improvements in diastolic dysfunction as assessed by changes in E/e′. In contrast, no such associations were present for serum PIIINP and galectin-3. These findings support PICP as the marker of choice to assess cardiac fibrosis and response to spironolactone.

References

1. Kobayashi M, Huttin O, Magnusson M, Ferreira JP, Bozec E, Huby AC, Preud'homme G, Duarte K, Lamiral Z, Dalleau K, Bresso E, Smaïl-Tabbone M, Devignes MD, Nilsson PM, Leosdottir M, Boivin JM, Zannad F, Rossignol P and Girerd N. Machine Learning-Derived Echocardiographic Phenotypes Predict Heart Failure Incidence in Asymptomatic Individuals. *JACC Cardiovascular imaging*. 2021.

2. Shah AM, Claggett B, Kitzman D, Biering-Sørensen T, Jensen JS, Cheng S, Matsushita K, Konety S, Folsom AR, Mosley TH, Wright JD, Heiss G and Solomon SD. Contemporary Assessment of Left Ventricular Diastolic Function in Older Adults: The Atherosclerosis Risk in Communities Study. *Circulation*. 2017;135:426-439.

3. López B, González A, Ravassa S, Beaumont J, Moreno MU, San José G, Querejeta R and Díez J. Circulating Biomarkers of Myocardial Fibrosis: The Need for a Reappraisal. *Journal of the American College of Cardiology*. 2015;65:2449-56.

4. Calvier L, Martinez-Martinez E, Miana M, Cachofeiro V, Rousseau E, Sádaba JR, Zannad F, Rossignol P and López-Andrés N. The impact of galectin-3 inhibition on aldosterone-induced cardiac and renal injuries. *JACC Heart Fail*. 2015;3:59-67.

5. Lopez-Andrès N, Rossignol P, Iraqi W, Fay R, Nuée J, Ghio S, Cleland JG, Zannad F and Lacolley P. Association of galectin-3 and fibrosis markers with long-term cardiovascular outcomes in patients with heart failure, left ventricular dysfunction, and dyssynchrony: insights from the CARE-HF (Cardiac Resynchronization in Heart Failure) trial. *Eur J Heart Fail*. 2012;14:74-81.

6. Cleland JGF, Pellicori P and González A. A novel treatment for heart failure targets myocardial fibrosis. *Nature medicine*. 2021;27:1343-1344.

7. López B, González A and Díez J. Circulating biomarkers of collagen metabolism in cardiac diseases. *Circulation*. 2010;121:1645-54.

8. Eschalier R, Rossignol P, Kearney-Schwartz A, Adamopoulos C, Karatzidou K, Fay R, Mandry D, Marie PY and Zannad F. Features of cardiac remodeling, associated with blood pressure and fibrosis biomarkers, are frequent in subjects with abdominal obesity. *Hypertension*. 2014;63:740-6.

9. López B, Ravassa S, Moreno MU, José GS, Beaumont J, González A and Díez J. Diffuse myocardial fibrosis: mechanisms, diagnosis and therapeutic approaches. *Nature reviews Cardiology*. 2021;18:479-498.

10. Zile MR, Jhund PS, Baicu CF, Claggett BL, Pieske B, Voors AA, Prescott MF, Shi V, Lefkowitz M, McMurray JJ and Solomon SD. Plasma Biomarkers Reflecting Profibrotic Processes in Heart Failure With a Preserved Ejection Fraction: Data From the Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction Study. *Circulation Heart failure*. 2016;9.

11. Cunningham JW, Claggett BL, O'Meara E, Prescott MF, Pfeffer MA, Shah SJ, Redfield MM, Zannad F, Chiang L-M, Rizkala AR, Shi VC, Lefkowitz MP, Rouleau J, McMurray JJV, Solomon SD and Zile MR. Effect of Sacubitril/Valsartan on Biomarkers of Extracellular Matrix Regulation in Patients With HFpEF. *Journal of the American College of Cardiology*. 2020;76:503-514.

12. Rockey DC, Bell PD and Hill JA. Fibrosis--a common pathway to organ injury and failure. *N Engl J Med*. 2015;372:1138-49.

13. Weiskirchen R, Weiskirchen S and Tacke F. Organ and tissue fibrosis: Molecular signals, cellular mechanisms and translational implications. *Molecular aspects of medicine*. 2019;65:2-15.

14. Rockey DC, Bell PD and Hill JA. Fibrosis — A Common Pathway to Organ Injury and Failure. *New England Journal of Medicine*. 2015;372:1138-1149.

15. Cleland JGF, Ferreira JP, Mariottoni B, Pellicori P, Cuthbert J, Verdonschot JAJ, Petutschnigg J, Ahmed FZ, Cosmi F, Brunner La Rocca HP, Mamas MA, Clark AL, Edelmann F, Pieske B, Khan J, McDonald K, Rouet P, Staessen JA, Mujaj B, González A, Diez J, Hazebroek M,

Heymans S, Latini R, Grojean S, Pizard A, Girerd N, Rossignol P, Collier TJ and Zannad F. 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. *European heart journal*. 2021;42:684-696.

16. Pellicori P, Ferreira JP, Mariottoni B, Brunner-La Rocca HP, Ahmed FZ, Verdonschot J, Collier T, Cuthbert JJ, Petutschnigg J, Mujaj B, Girerd N, González A, Clark AL, Cosmi F, Staessen JA, Heymans S, Latini R, Rossignol P, Zannad F and Cleland JGF. 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. *European journal of heart failure*. 2020.

17. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW and Van Lente F. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med*. 2006;145:247-54.

18. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF, 3rd, Dokainish H, Edvardsen T, Flachskampf FA, Gillebert TC, Klein AL, Lancellotti P, Marino P, Oh JK, Popescu BA and Waggoner AD. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. 2016;29:277-314.

19. Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, Solomon SD, Louie EK and Schiller NB. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. *J Am Soc Echocardiogr*. 2010;23:685-713; quiz 786-8.

20. Frikha Z, Girerd N, Huttin O, Courand PY, Bozec E, Olivier A, Lamiral Z, Zannad F and Rossignol P. Reproducibility in echocardiographic assessment of diastolic function in a population based study (the STANISLAS Cohort study). *PLoS One*. 2015;10:e0122336.

21. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W and Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*. 2015;28:1-39.e14.

22. Zannad F and Ferreira JP. Is Sacubitril/Valsartan Antifibrotic? *Journal of the American College of Cardiology*. 2019;73:807-809.

23. Ferreira JP, Rossignol P, Pizard A, Machu J-L, Collier T, Girerd N, Huby A-C, Gonzalez A, Diez J, López B, Sattar N, Cleland JG, Sever PS and Zannad F. Potential spironolactone effects on collagen metabolism biomarkers in patients with uncontrolled blood pressure. *Heart*. 2019;105:307-314.

24. Zile MR, Jhund PS, Baicu CF, Claggett BL, Pieske B, Voors AA, Prescott MF, Shi V, Lefkowitz M, McMurray JJ, Solomon SD and Prospective Comparison of AWARBoMoHFWPEFI. Plasma Biomarkers Reflecting Profibrotic Processes in Heart Failure With a Preserved Ejection Fraction: Data From the Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction Study. *Circulation Heart failure*. 2016;9.

25. Ravassa S, Trippel T, Bach D, Bachran D, González A, López B, Wachter R, Hasenfuss G, Delles C, Dominiczak AF, Pieske B, Díez J and Edelmann F. 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. *European journal of heart failure*. 2018;20:1290-1299.

26. Kosmala W, Jedrzejuk D, Derzhko R, Przewlocka-Kosmala M, Mysiak A and Bednarek-Tupikowska G. Left ventricular function impairment in patients with normal-weight obesity: contribution of abdominal fat deposition, profibrotic state, reduced insulin sensitivity, and proinflammatory activation. *Circulation Cardiovascular imaging*. 2012;5:349-56.

27. Zannad F and Rossignol P. Cardiorenal Syndrome Revisited. *Circulation*. 2018;138:929-944.

28. Romero-González G, González A, López B, Ravassa S and Díez J. Heart failure in chronic kidney disease: the emerging role of myocardial fibrosis. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. 2020.

29. Soylemezoglu O, Wild G, Dalley AJ, MacNeil S, Milford-Ward A, Brown CB and el Nahas AM. Urinary and serum type III collagen: markers of renal fibrosis. *Nephrol Dial Transplant*. 1997;12:1883-9.

30. Shah RV, Chen-Tournoux AA, Picard MH, van Kimmenade RR and Januzzi JL. Galectin-3, cardiac structure and function, and long-term mortality in patients with acutely decompensated heart failure. *Eur J Heart Fail*. 2010;12:826-32.

31. Aguilar D, Sun C, Hoogeveen RC, Nambi V, Selvin E, Matsushita K, Saeed A, McEvoy JW, Shah AM, Solomon SD, Boerwinkle E and Ballantyne CM. Levels and Change in Galectin-3 and Association With Cardiovascular Events: The ARIC Study. *J Am Heart Assoc*. 2020;9:e015405.

32. Ho JE, Liu C, Lyass A, Courchesne P, Pencina MJ, Vasan RS, Larson MG and Levy D. Galectin-3, a marker of cardiac fibrosis, predicts incident heart failure in the community. *Journal of the American College of Cardiology*. 2012;60:1249-56.

33. Martínez-Martínez E, Calvier L, Fernández-Celis A, Rousseau E, Jurado-López R, Rossoni LV, Jaisser F, Zannad F, Rossignol P, Cachofeiro V and López-Andrés N. Galectin-3 blockade inhibits cardiac inflammation and fibrosis in experimental hyperaldosteronism and hypertension. *Hypertension*. 2015;66:767-75.

34. Querejeta R, López B, González A, Sánchez E, Larman M, Martínez Ubago JL and Díez J. Increased collagen type I synthesis in patients with heart failure of hypertensive origin: relation to myocardial fibrosis. *Circulation*. 2004;110:1263-8.

35. Querejeta R, Varo N, López B, Larman M, Artiñano E, Etayo JC, Martínez Ubago JL, Gutierrez-Stampa M, Emparanza JI, Gil MJ, Monreal I, Mindán JP and Díez J. Serum carboxy-terminal propeptide of procollagen type I is a marker of myocardial fibrosis in hypertensive heart disease. *Circulation*. 2000;101:1729-35.

36. Raafs AG, Verdonschot JAJ, Henkens M, Adriaans BP, Wang P, Derks K, Abdul Hamid MA, Knackstedt C, van Empel VPM, Díez J, Brunner-La Rocca HP, Brunner HG, González A, Bekkers S, Heymans SRB and Hazebroek MR. The combination of carboxy-terminal propeptide of procollagen type I blood levels and late gadolinium enhancement at cardiac magnetic resonance provides additional prognostic information in idiopathic dilated cardiomyopathy - A multilevel assessment of myocardial fibrosis in dilated cardiomyopathy. *Eur J Heart Fail*. 2021;23:933-944.

37. Weber KT. Cardiac interstitium in health and disease: the fibrillar collagen network. *J Am Coll Cardiol*. 1989;13:1637-52.

38. Hulmes David JS. Roles of the procollagen C-propeptides in health and disease. *Essays in Biochemistry*. 2019;63:313-323.

39. López B, Querejeta R, Varo N, González A, Larman M, Martínez Ubago JL and Díez J. Usefulness of serum carboxy-terminal propeptide of procollagen type I in assessment of the cardioreparative ability of antihypertensive treatment in hypertensive patients. *Circulation*. 2001;104:286-91.

40. López B, Querejeta R, González A, Sánchez E, Larman M and Díez J. Effects of loop diuretics on myocardial fibrosis and collagen type I turnover in chronic heart failure. *Journal of the American College of Cardiology*. 2004;43:2028-35.

41. Ravassa S, López B, Ferreira JP, Girerd N, Bozec E, Pellicori P, Mariottoni B, Cosmi F, Hazebroek M, Verdonschot JAJ, Cuthbert J, Petutschnigg J, Moreno MU, Heymans S, Staessen JA, Pieske B, Edelmann F, Clark AL, Cleland JGF, Zannad F, Díez J and González A. 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. *European journal of heart failure*. 2022;24:321-331.

42. Zannad F, Alla F, Dousset B, Perez A and 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). Rales Investigators. *Circulation*. 2000;102:2700-6.

43. Díez J, González A and Kovacic JC. Myocardial Interstitial Fibrosis in Nonischemic Heart Disease, Part 3/4: JACC Focus Seminar. *Journal of the American College of Cardiology*. 2020;75:2204-2218.

44. Pellicori P, Ferreira JP, Mariottoni B, Brunner-La Rocca HP, Ahmed FZ, Verdonschot J, Collier T, Cuthbert JJ, Petutschnigg J, Mujaj B, Girerd N, González A, Clark AL, Cosmi F, Staessen JA, Heymans S, Latini R, Rossignol P, Zannad F and Cleland JGF. 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. *European journal of heart failure*. 2020;22:1711-1723.

45. Aguinis H. Statistical Power with Moderated Multiple Regression in Management Research. *Journal of Management*. 1995;21:1141-1158.

46. Pellicori P and Cleland JG. Structural heart disease, cardiac dysfunction and heart failure: the ambiguity of a definition. *European journal of preventive cardiology*. 0:2047487320937135.

Graphical abstract

PICP, procollagen type I C-terminal propeptide; PIIINP, procollagen type III N-terminal propeptide; LV, left ventricular; LA, left atrial

Graphical abstract

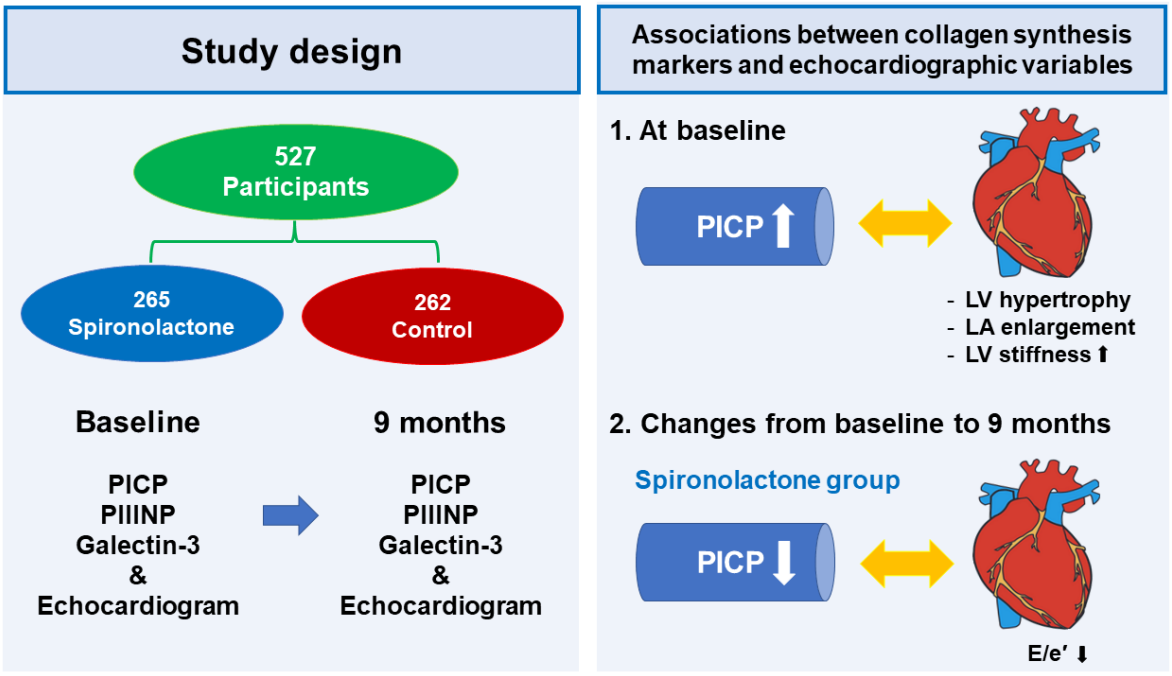


Table 1. Baseline characteristics of subjects according to tertiles of serum concentrations of PICP and PIIINP

Values are expressed as mean ±SD, n (%) or median (25th to 75th percentile)

PIIINP, procollagen type-III N-terminal propeptide; PICP, procollagen type-I C-terminal propeptide; CITP, collagen type-1 C-terminal telopeptide; MMP-1, matrix metalloproteinase-1; BMI, body mass index; PCI, percutaneous coronary intervention; CBAG, coronary artery bypass graft; TIA, transient ischemic attack; COPD, chronic obstructive pulmonary disease; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; BP, blood pressure; eGFR, estimated glomerular filtration rate; NT-proBNP, amino-terminal B-type natriuretic peptide; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVMi, left ventricular mass index; LVH, left ventricular hypertrophy; LAVi, left atrial volume index; TAPSE, tricuspid annular plane systolic excursion.

Table 2. Multinomial logistic model for clinical factors associated with serum concentrations of baseline PICP, PIIINP and galectin -3

OR, odd ratio; CI, confidence interval; Other abbreviations are presented in *Table 1*.

Patients with tertile 1 of serum collagen markers as a reference.

Table 3. Associations between baseline serum PICP, PIIINP, galectin-3 and baseline echocardiographic variables in multivariable logistic regression analyses

Each model for abnormalities of echocardiographic function and structure was adjusted for clinical confounders selected in the results of Table 2.

OR, odd ratio; CI, confidence interval; Other abbreviations are presented in *Table 1*.

Dilated LVEDVi was defined by an LVEDVi>74ml/m² in men or >61ml/m² in women, decreased LVEF by an LVEF<52% in men or <54% in women, LV hypertrophy by a LVMi>115g/m² in men or >95g/m² in women and decreased e′ by a septal e′ <7cm/sec or lateral e′<10cm/sec.

Table 4. Associations between changes in PICP, PIIINP and galectin 3, and changes in echocardiographic variables in multivariable linear regression analyses

*PICP, PIIINP and galectin-3 were transformed using a logarithm function.

Each model was adjusted for clinical confounders selected in the results of Table 2 plus baseline collagen-related marker and baseline echocardiographic variable.

CI, confidence interval; Other abbreviations are presented in *Table 1*.

Table 1. Baseline characteristics of subjects according to tertiles of serum concentrations of PICP and PIIINP

	PICP T1 (<71.9µg/L) (N=172)	PICP T2 (71.9-91.2µg/L) (N=172)	PICP T3 (>91.2µg/L) (N=172)	p-value	PIIINP T1 (<3.4µg/L) (N=171)	PIIINP T2 (3.4-4.6µg/L) (N=170)	PIIINP T3 (>4.6µg/L) (N=171)	p-value
Collagen markers								
PIIINP, µg/L	3.6 (3.0 - 4.6)	3.9 (3.0 - 5.0)	4.3 (3.3 - 5.4)	**<0.001**	2.8 (2.4 - 3.1)	3.9 (3.6 - 4.3)	5.5 (5.0 - 6.5)	**<0.001**
PICP, µg/L	60.5 (53.5 - 65.1)	80.6 (75.8 - 84.8)	105.9 (97.1 - 121.7)	**<0.001**	77.3 (62.6 - 93.1)	77.2 (64.3 - 95.1)	86.4 (70.8 - 104.8)	**<0.001**
CITP, µg/L	3.4 (2.6 - 4.2)	3.7 (2.8 - 4.7)	4.3 (3.2 - 5.9)	**<0.001**	3.2 (2.5 - 4.1)	3.4 (2.8 - 4.5)	4.7 (3.6 - 6.2)	**<0.001**
PICP/CITP ratio	17.3 (13.4 - 22.6)	22.0 (17.3 - 28.8)	25.4 (18.1 - 34.5)	**<0.001**	24.0 (18.5 - 30.5)	21.5 (16.7 - 28.9)	18.1 (13.5 - 24.0)	**<0.001**
CITP/MMP-1 ratio	1.21 (0.70 - 2.02)	1.26 (0.83 - 1.95)	1.36 (0.88 - 2.27)	0.08	1.04 (0.65 - 1.85)	1.32 (0.85 - 1.98)	1.53 (0.89 - 2.60)	**<0.001**
Galectin-3, µg/L	16.0 (13.6 - 19.4)	15.6 (12.9 - 19.2)	16.5 (13.9 - 20.7)	0.18	15.9 (13.1 - 19.5)	15.4 (13.2 - 19.4)	17.0 (14.1 - 21.0)	**0.019**
Age, years	73 (68 - 77)	72 (69 - 78)	74 (69 - 79)	0.38	72 (68 - 78)	73 (69 - 79)	74 (70 - 79)	**0.032**
Women, N (%)	39 (22.5 %)	36 (20.9 %)	56 (32.6 %)	**0.027**	53 (31.0 %)	36 (21.2 %)	41 (24.0 %)	0.10
BMI, kg/m²	28 (25 - 31)	28 (26 - 32)	28 (25 - 32)	0.33	27 (24 - 30)	28 (26 - 32)	28 (26 - 32)	**<0.001**
Waist circumference, cm	101 (95 - 110)	101 (94 - 110)	103 (95 - 112)	0.68	99 (92 - 107)	103 (96 - 112)	104 (97 - 112)	**<0.001**
Smoking, N (%)	10 (5.8 %)	10 (5.8 %)	22 (12.8 %)	**0.023**	16 (9.4 %)	15 (8.8 %)	11 (6.4 %)	0.58
Medical history, N (%)								
Hypertension	137 (79.2 %)	131 (76.2 %)	137 (79.7 %)	0.69	123 (71.9 %)	138 (81.2 %)	141 (82.5 %)	**0.035**
Diabetes	78 (45.1 %)	69 (40.1 %)	62 (36.0 %)	0.23	64 (37.4 %)	75 (44.1 %)	71 (41.5 %)	0.45
Coronary artery disease	126 (72.8 %)	132 (76.7 %)	115 (66.9 %)	0.12	125 (73.1 %)	116 (68.2 %)	125 (73.1 %)	0.52
Myocardial infarction	70 (40.5 %)	78 (45.3 %)	63 (36.6 %)	0.26	79 (46.2 %)	60 (35.3 %)	68 (39.8 %)	0.12
Stroke or TIA	5 (2.9 %)	7 (4.1 %)	15 (8.7 %)	**0.037**	4 (2.3 %)	11 (6.5 %)	13 (7.6 %)	0.079
COPD	14 (8.1 %)	9 (5.2 %)	9 (5.2 %)	0.44	11 (6.4 %)	14 (8.2 %)	7 (4.1 %)	0.29
Treatments, N (%)								
ACEi or ARB	135 (78.0 %)	140 (81.4 %)	135 (78.5 %)	0.71	135 (78.9 %)	134 (78.8 %)	136 (79.5 %)	0.99
Beta-blocker	125 (72.3 %)	123 (71.5 %)	112 (65.1 %)	0.29	122 (71.3 %)	116 (68.2 %)	117 (68.4 %)	0.78
CCB	29 (16.8 %)	38 (22.1 %)	39 (22.7 %)	0.32	34 (19.9 %)	38 (22.4 %)	36 (21.1 %)	0.86
Lipid-lowering therapy	145 (83.8 %)	143 (83.1 %)	138 (80.2 %)	0.65	141 (82.5 %)	137 (80.6 %)	144 (84.2 %)	0.68
Clinical profile								
Systolic BP, mmHg	141 (128 - 155)	139 (127 - 156)	140 (128 - 154)	0.89	139 (126 - 153)	141 (130 - 157)	141 (126 - 157)	0.17
Diastolic BP, mmHg	78 (71 - 86)	78 (72 - 85)	78 (71 - 84)	0.78	78 (69 - 84)	78 (73 - 85)	78 (71 - 84)	0.33
Heart rate, bpm	61 (55 - 68)	60 (54 - 67)	61 (55 - 67)	0.58	62 (56 - 68)	60 (54 - 66)	60 (55 - 67)	0.37
Number of shuttles completed	45 (31 - 63)	52 (35 - 66)	49 (30 - 73)	0.22	48 (33 - 66)	54 (37 - 70)	45 (26 - 64)	0.10
Laboratory data								
Hemoglobin, g/dL	14.0 (13.1 - 14.7)	14.2 (13.2 - 15.0)	13.9 (12.9 - 14.9)	0.35	14.0 (13.1 - 14.6)	14.0 (13.2 - 14.9)	14.0 (12.9 - 15.1)	0.74
Anemia, N (%)	29 (16.8 %)	24 (14.0 %)	27 (15.8 %)	0.78	20 (11.8 %)	25 (14.7 %)	35 (20.5 %)	0.083
Sodium, mmol/L	139 (137 - 140)	140 (138 - 142)	140 (138 - 142)	**<0.001**	139 (137 - 141)	139 (138 - 141)	140 (138 - 142)	**0.021**
Potassium, mmol/L	4.3 (4.1 - 4.5)	4.3 (4.1 - 4.6)	4.3 (4.1 - 4.6)	0.81	4.4 (4.1 - 4.6)	4.3 (4.1 - 4.6)	4.3 (4.0 - 4.5)	**0.033**
eGFR, mL/min/1.73m²	72 (61 - 86)	68 (60 - 82)	70 (59 - 81)	**0.012**	70 (60 - 84)	72 (61 - 87)	69 (57 - 80)	**0.012**
NT-proBNP, pg/ml	172 (131 – 288)	187 (122 – 323)	268 (183 – 505)	**<0.001**	200 (136 – 336)	179 (131 – 299)	278 (157 – 497)	**<0.001**
Echocardiographic variables								
LVEDVi, mL/m²	42 (36 - 49)	40 (35 - 47)	41 (36 - 50)	0.18	43 (36 - 49)	41 (35 - 49)	41 (35 - 49)	0.40
LVEF, %	64 (58 - 68)	63 (57 - 67)	61 (56 - 66)	0.15	63 (56 - 68)	62 (58 - 66)	63 (57 - 66)	0.67

LVMi, g/m²	92 (77 - 108)	93 (84 - 112)	99 (83 - 120)	**<0.001**	92 (78 - 106)	94 (83 - 115)	98 (81 - 115)	**0.028**
Men	92 (78 - 110)	95 (85 - 114)	100 (83 - 122)	**0.005**	93 (79 - 110)	96 (84 - 115)	97 (81 - 116)	0.23
Women	87 (68 - 101)	89 (79 - 106)	97 (82 - 112)	**0.007**	87 (74 - 103)	90 (80 - 103)	101 (82 - 114)	**0.043**
LAVi, mL/m²	30 (25 - 34)	30 (26 - 37)	32 (26 - 37)	**0.039**	30 (26 - 35)	30 (26 - 36)	32 (26 - 37)	0.70
E/A ratio	0.8 (0.7 - 1.0)	0.8 (0.7 - 1.0)	0.8 (0.7 - 1.0)	0.19	0.8 (0.7 - 1.0)	0.8 (0.7 - 1.0)	0.8 (0.7 - 1.0)	0.56
Lateral e′, cm/sec	8.0 (7.0 - 10.0)	8.2 (6.6 - 10.0)	8.2 (7.0 - 10.0)	0.85	8.3 (7.0 - 10.0)	8.8 (6.6 - 10.5)	8.0 (7.0 - 10.0)	0.28
Septal e′, cm/sec	5.5 (4.8 - 7.2)	5.7 (4.8 - 7.0)	5.9 (5.0 - 6.8)	0.74	5.7 (4.9 - 7.1)	5.7 (4.8 - 7.1)	5.6 (4.8 - 6.6)	0.24
Average e′, cm/sec	7.1 (6.2 - 8.7)	7.2 (5.9 - 8.4)	7.3 (6.0 - 8.4)	0.83	7.2 (6.0 - 8.3)	7.3 (5.8 - 8.6)	7.0 (6.1 - 8.4)	0.30
Average E/e′ ratio	9.0 (7.3 - 11.5)	9.6 (7.9 - 11.6)	9.4 (7.6 - 11.5)	0.46	9.0 (7.4 - 11.4)	8.9 (7.5 - 11.6)	9.7 (7.8 - 11.6)	0.32
TAPSE, cm	22.2 (16.6 - 26.0)	22.8 (18.2 - 27.7)	20.6 (16.6 - 25.4)	0.11	22.6 (17.8 - 27.8)	21.9 (16.8 - 26.3)	21.1 (17.5 - 25.9)	0.47

Table 2. Multinomial logistic model for clinical factors associated with serum concentrations of baseline PICP, PIIINP and galectin -3

PICP model	**Tertile 2 (71.9-91.2µg/L)**		**Tertile 3 (>91.2µg/L)**	
	OR (95%CI)	**p-value**	**OR (95%CI)**	**p-value**
Women	1.01 (0.58 - 1.75)	0.98	1.95 (1.14 - 3.31)	**0.01**
BMI (per 5kg/m²)	1.14 (0.90 - 1.43)	0.29	1.35 (1.07 - 1.71)	**0.01**
Number of shuttles completed	1.01 (0.99 - 1.02)	0.10	1.01 (1.002 - 1.02)	**0.02**
Smoke	1.17 (0.46 - 3.00)	0.74	3.25 (1.41 - 7.49)	**0.006**
eGFR (per 5ml/min/1.73m²)	0.91 (0.85 - 0.98)	**0.01**	0.92 (0.86 - 0.99)	**0.03**
PIIINP model	**Tertile 2 (3.4-4.6µg/L)**		**Tertile 3 (>4.6µg/L)**	
	OR (95%CI)	**p-value**	**OR (95%CI)**	**p-value**
Age (per 5years)	1.25 (1.03 - 1.51)	**0.02**	1.33 (1.10 - 1.60)	**0.003**
Women	0.54 (0.33 - 0.91)	**0.02**	0.59 (0.36 - 0.99)	**0.046**
BMI (per 5kg/m²)	1.55 (1.21 - 1.97)	**<0.001**	1.73 (1.35 - 2.21)	**<0.001**
Potassium	0.64 (0.33 - 1.24)	0.19	0.40 (0.21 - 0.77)	**0.006**
Galectin-3 model	**Tertile 2 (14.5-18.3µg/L)**		**Tertile 3 (>18.3µg/L)**	
	OR (95%CI)	**p-value**	**OR (95%CI)**	**p-value**
Age (per 5years)	1.27 (1.06 – 1.52)	**0.009**	1.36 (1.13 – 1.64)	**0.001**
Diabetes	1.45 (0.92 – 2.31)	0.11	2.67 (1.65 – 4.30)	**<0.001**
eGFR (per 5ml/min/1.73m²)	0.97 (0.90 – 1.04)	0.40	0.81 (0.74 – 0.87)	**<0.001**

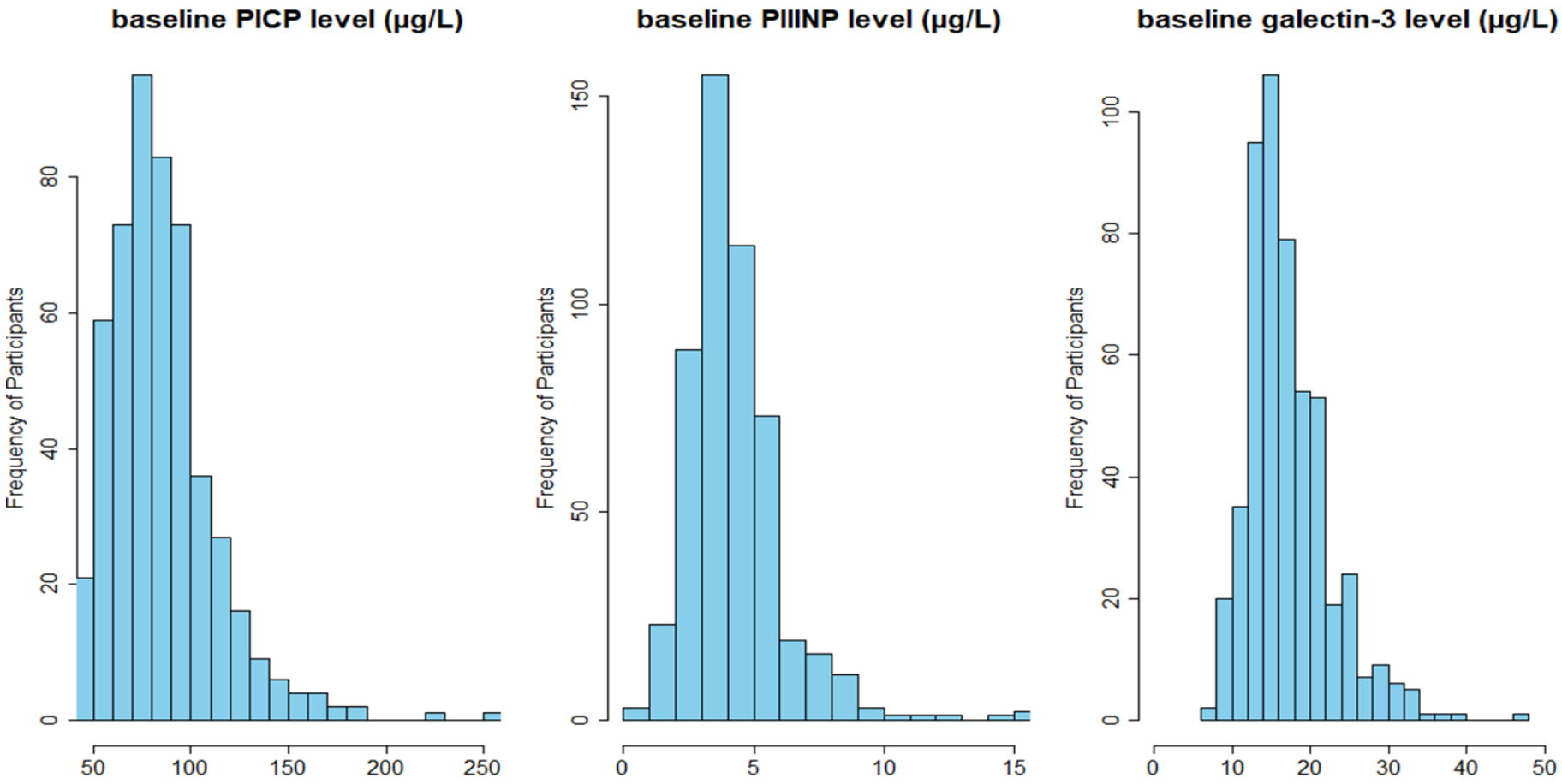
Table 3. Associations between baseline serum PICP, PIIINP, galectin-3 and baseline echocardiographic variables in multivariable logistic regression analyses

PICP model	Tertile 2 (71.9-91.2µg/L)		Tertile 3 (>91.2µg/L)	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Dilated LVEDVi	0.20 (0.01 - 1.44)	0.16	0.67 (0.12 - 3.30)	0.62
Decreased LVEF	1.03 (0.47 - 2.25)	0.95	1.19 (0.54 - 2.64)	0.66
LV hypertrophy	1.32 (0.76 - 2.31)	0.33	1.89 (1.08 - 3.32)	**0.026**
LAVi>34mL/m²	1.25 (0.75 - 2.08)	0.39	2.01 (1.21 - 3.37)	**0.007**
Decreased e′	1.32 (0.69 - 2.52)	0.40	2.32 (1.12 - 5.06)	**0.028**
Average E/e′>14	1.15 (0.50 - 2.72)	0.74	1.52 (0.68 - 3.53)	0.31
TAPSE<16cm	0.52 (0.28 - 0.95)	**0.037**	0.85 (0.47 - 1.53)	0.59
PIIINP model	**Tertile 2 (3.4-4.6µg/L)**		**Tertile 3 (>4.6µg/L)**	
	OR (95%CI)	**p-value**	**OR (95%CI)**	**p-value**
Dilated LVEDVi	0.53 (0.07 - 2.85)	0.48	0.53 (0.07 - 2.95)	0.48
Decreased LVEF	0.90 (0.40 - 2.00)	0.80	1.28 (0.60 - 2.75)	0.53
LV hypertrophy	1.16 (0.68 - 1.99)	0.58	1.20 (0.70 - 2.03)	0.51
LAVi>34mL/m²	1.27 (0.78 - 2.09)	0.34	1.59 (0.98 - 2.60)	0.06
Decreased e′	0.81 (0.42 - 1.55)	0.52	1.71 (0.80 - 3.81)	0.18
Average E/e′>14	1.48 (0.64 - 3.52)	0.37	1.97 (0.89 - 4.56)	0.10
TAPSE<16cm	0.95 (0.52 - 1.70)	0.85	0.84 (0.45 - 1.54)	0.57
Galectin-3 model	**Tertile 2 (14.5-18.3µg/L)**		**Tertile 3 (>18.3µg/L)**	
	OR (95%CI)	**p-value**	**OR (95%CI)**	**p-value**
Dilated LVEDVi	1.12 (0.20 - 6.24)	0.90	0.76 (0.09 - 5.23)	0.78
Decreased LVEF	0.84 (0.40 - 1.72)	0.63	0.49 (0.20 - 1.13)	0.10
LV hypertrophy	1.77 (1.06 - 3.00)	**0.03**	1.39 (0.80 - 2.42)	0.25
LAVi>34mL/m²	0.79 (0.49 - 1.28)	0.33	0.82 (0.49 - 1.35)	0.43
Decreased e′	0.98 (0.52 - 1.87)	0.95	1.63 (0.77 - 3.57)	0.21
Average E/e′>14	0.85 (0.36 - 2.01)	0.70	1.18 (0.52 - 2.72)	0.70
TAPSE<16cm	1.56 (0.87 - 2.82)	0.14	0.79 (0.41 - 1.54)	0.49

Table 4. Associations between changes in PICP, PIIINP and galectin 3, and changes in echocardiographic variables in multivariable linear regression analyses

	Change in PICP*				**Change in PIIINP***				**Change in Galectin-3***			
	Placebo		**Spironolactone**		**Placebo**		**Spironolactone**		**Placebo**		**Spironolactone**	
	beta (95%CI)	**p-value**	**beta (95%CI)**	**P-value**	**beta (95%CI)**	**p-value**	**beta (95%CI)**	**p-value**	**beta (95%CI)**	**p-value**	**beta (95%CI)**	**p-value**
Change in LVEDVi	2.05 (-0.18 - 4.27)	0.07	-0.09 (-2.05 - 1.86)	0.93	-0.43 (-1.70 - 0.84)	0.50	-0.20 (-1.54 - 1.15)	0.78	0.11 (-2.53 - 2.75)	0.93	0.14 (-2.62 - 2.89)	0.92
Change in LVEF	0.05 (-2.15 - 2.26)	0.96	0.45 (-1.49 - 2.38)	0.65	-0.30 (-1.53 - 0.93)	0.63	0.89 (-0.51 - 2.30)	0.21	-1.50 (-4.13 - 1.14)	0.26	0.07 (-2.78 - 2.92)	0.96
Change in LVMi	2.49 (-2.74 - 7.72)	0.35	-1.85 (-6.65 - 2.96)	0.45	0.33 (-2.62 - 3.28)	0.83	-1.55 (-5.09 - 1.99)	0.39	-0.46 (-6.97 - 6.06)	0.89	-2.93 (-9.75 - 3.89)	0.40
Change in LAVi	0.93 (-1.51 - 3.37)	0.45	1.66 (-0.39 - 3.71)	0.11	0.11 (-1.28 - 1.51)	0.87	0.88 (-0.57 - 2.33)	0.23	1.75 (-1.35 - 4.85)	0.27	-5.69 (-8.58 - -2.79)	**<0.001**
Change in e′ mean	0.05 (-0.64 - 0.73)	0.89	-0.36 (-0.88 - 0.17)	0.18	-0.14 (-0.52 - 0.25)	0.49	0.19 (-0.21 - 0.58)	0.35	0.39 (-0.46 - 1.23)	0.37	-0.05 (-0.81 - 0.72)	0.90
Change in E/e′ mean	0.80 (-0.31 - 1.92)	0.16	1.07 (0.28 - 1.86)	**0.008**	0.65 (-0.02 - 1.32)	0.06	0.03 (-0.58 - 0.65)	0.91	-0.75 (-2.20 - 0.70)	0.31	-0.85 (-2.06 - 0.36)	0.17
Change in TAPSE	-1.21 (-3.43 - 1.02)	0.29	0.83 (-1.18 - 2.84)	0.42	-0.94 (-2.16 - 0.28)	0.13	0.94 (-0.45 - 2.32)	0.19	-3.54 (-6.09 - -0.99)	**0.007**	-1.06 (-4.14 - 2.02)	0.50

Supplemental Figure 1. Histogram of the distribution of serum concentration of baseline PICP, PIIINP and galectin-3



Supplemental Table 1. Correlation matrix among collagen-related markers

	PICP		PIIINP	
	r	**p-value**	**r**	**p-value**
PIIINP	0.195	<0.001	—	—
Galectin-3	0.052	0.24	0.100	0.02

Supplemental Table 2. Baseline characteristics of subjects according to tertiles of serum concentrations of galectin-3

	Galectin-3 T1 (<14.5µg/L) (N=173)	Galectin-3 T2 (14.5-18.3µg/L) (N=172)	Galectin-3 T3 (>18.3µg/L) (N=173)	p-value
Collagen markers				
PIIINP, µg/L	3.7 (3.0 - 4.8)	4.0 (3.1 - 5.0)	4.1 (3.2 - 5.0)	0.10
PICP, µg/L	78.3 (65.8 - 92.1)	80.8 (65.7 - 100.5)	81.0 (64.6 - 100.1)	0.32
CITP, µg/L	3.2 (2.7 - 4.1)	3.5 (2.7 - 4.7)	4.4 (3.5 - 6.0)	**<0.001**
PICP/CITP ratio	23.9 (17.5 - 30.4)	22.7 (16.7 - 30.3)	18.2 (14.1 - 24.0)	**<0.001**
CITP/MMP-1 ratio	1.17 (0.77 - 1.98)	1.39 (0.74 - 2.15)	1.31 (0.87 - 2.20)	0.36
Galectin-3, µg/L	12.7 (11.3 - 13.5)	16.1 (15.3 - 17.0)	21.5 (19.7 - 25.0)	**<0.001**
Age, years	71 (67 - 76)	73 (69 - 79)	75 (70 - 80)	**<0.001**
Women, N (%)	37 (21.4 %)	45 (26.2 %)	50 (28.9 %)	0.27
BMI, kg/m²	28 (25 - 31)	28 (25 - 31)	28 (26 - 32)	0.68
Waist circumference, cm	101 (94 - 110)	101 (94 - 110)	103 (95 - 111)	0.52
Smoking, N (%)	10 (5.8 %)	14 (8.1 %)	18 (10.4 %)	0.29
Medical history, N (%)				
Hypertension	125 (72.3 %)	135 (78.5 %)	148 (85.5 %)	**0.01**
Diabetes	54 (31.2 %)	67 (39.0 %)	89 (51.4 %)	**<0.001**
Coronary artery disease	141 (81.5 %)	126 (73.3 %)	105 (60.7 %)	**<0.001**
Myocardial infarction	84 (48.6 %)	64 (37.2 %)	62 (35.8 %)	**0.03**
Stroke or TIA	9 (5.2 %)	13 (7.6 %)	6 (3.5 %)	0.24
COPD	14 (8.1 %)	9 (5.2 %)	9 (5.2 %)	0.44
Treatments, N (%)				
ACEi or ARB	129 (74.6 %)	135 (78.5 %)	147 (85.0 %)	0.054
Beta-blocker	125 (72.3 %)	119 (69.2 %)	115 (66.5 %)	0.51
CCB	35 (20.2 %)	39 (22.7 %)	35 (20.2 %)	0.81
Lipid-lowering therapy	153 (88.4 %)	147 (85.5 %)	127 (73.4 %)	**<0.001**
Clinical profile				
Systolic BP, mmHg	142 (129 - 155)	138 (126 - 153)	142 (126 - 157)	0.23
Diastolic BP, mmHg	78 (71 - 86)	78 (71 - 84)	78 (71 - 84)	0.70
Heart rate, bpm	59 (54 - 65)	62 (55 - 67)	63 (56 - 70)	**0.012**
Number of shuttles completed	54 (36 - 70)	49 (35 - 66)	44 (26 - 62)	0.13
Laboratory data				
Hemoglobin, g/dL	14.2 (13.3 - 15.0)	14.0 (13.2 - 14.8)	13.8 (12.8 - 14.6)	**0.004**
Anemia, N (%)	19 (11.0 %)	23 (13.5 %)	38 (22.1 %)	**0.011**
Sodium, mmol/L	139 (138 - 141)	139 (138 - 141)	140 (138 - 141)	0.63
Potassium, mmol/L	4.3 (4.1 - 4.6)	4.3 (4.1 - 4.5)	4.4 (4.0 - 4.6)	0.65
eGFR, mL/min/1.73m²	73 (63 - 87)	74 (60 - 86)	63 (52 - 77)	**<0.001**
NT-proBNP, pg/ml	184 (126 – 313)	207 (134 – 340)	251 (141 – 435)	**0.04**
Echocardiographic variables				
LVEDVi, mL/m²	43 (37 - 50)	41 (35 - 49)	41 (34 - 49)	0.16
LVEF, %	63 (58 - 67)	62 (57 - 66)	62 (56 - 67)	0.79
LVMi, g/m²	91 (79 - 103)	97 (83 - 116)	97 (82 - 113)	**0.047**
Men	92 (78 - 106)	100 (85 - 119)	97 (83 - 114)	**0.028**
Women	89 (81 - 100)	93 (80 - 108)	100 (75 - 111)	0.64
LAVi, mL/m²	31 (26 - 37)	29 (24 - 36)	31 (26 - 36)	0.35
E/A ratio	0.9 (0.7 - 1.1)	0.9 (0.7 - 1.0)	0.7 (0.7 - 1.0)	**0.028**
Lateral e′, cm/sec	9.0 (7.9 - 11.0)	8.0 (7.0 - 10.0)	8.0 (6.0 - 10.0)	**0.001**
Septal e′, cm/sec	5.9 (4.9 - 7.1)	5.8 (4.9 - 7.1)	5.5 (4.6 - 6.7)	0.067
Average e′, cm/sec	7.4 (6.4 - 8.7)	7.1 (6.0 - 8.3)	6.9 (5.7 - 8.3)	**0.001**
Average E/e′ ratio	8.9 (6.9 - 10.8)	9.3 (7.7 - 11.4)	9.6 (7.9 - 11.9)	**0.022**
TAPSE, cm	22.8 (17.7 - 27.5)	21.5 (16.0 - 26.3)	21.6 (17.1 - 25.9)	0.36

Supplemental Table 3. The association of collagen-related markers with left ventricular mass and left atrial volume variables

	PICP*		PIIINP*		Galectin-3*	
	adjusted-beta (95%CI)	**p-value**	**adjusted-beta (95%CI)**	**p-value**	**adjusted-beta (95%CI)**	**p-value**
LV mass	24.09 (12.68 - 35.50)	**<0.001**	2.20 (-6.05 - 10.44)	0.60	11.29 (-2.60 - 25.17)	0.11
LV mass/BSA	13.25 (7.42 - 19.07)	**<0.001**	1.48 (-2.70 - 5.66)	0.49	6.39 (-0.02 - 12.81)	0.051
LV mass/height	14.79 (8.11 - 21.46)	**<0.001**	1.57 (-3.24 - 6.38)	0.52	7.28 (-0.75 - 15.30)	0.08
LA volume	5.50 (1.78 - 9.22)	**0.004**	0.47 (-2.31 - 3.25)	0.74	0.09 (-4.18 - 4.36)	0.97
LA volume/BSA	2.98 (1.05 - 4.92)	**0.003**	0.17 (-1.26 - 1.61)	0.81	0.59 (-1.55 - 2.73)	0.59
LA volume/height	3.40 (1.22 - 5.58)	**0.002**	0.26 (-1.36 - 1.88)	0.75	0.42 (-2.06 - 2.89)	0.74

*PICP, PIIINP and galectin-3 were transformed using a logarithm function.

Respective model was adjusted for clinical confounders selected in *Table 2*.