

Differential biomarker expression in heart failure patients with and without mitral regurgitation: insights from BIOSTAT-CHF

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ABSTRACT (240 words)

Background: Mitral regurgitation (MR) frequently coexists with heart failure (HF).

Objectives: To better understand potential pathophysiological differences between patients with HF with or without moderate-severe MR, we compared differentially expressed circulating biomarkers between these two groups.

Methods: The Olink Proteomics® Multiplex Cardiovascular (CVD) -II, CVD-III, Immune Response and Oncology-II panels of 363 unique proteins from different pathophysiological domains were used to investigate the biomarker profiles of HF patients from index and validation cohorts of the BIOSTAT-CHF study stratified according to the presence of moderate-to-severe MR or no-mild MR.

Results: The index cohort included 888 patients (46%) with moderate-to-severe MR and 1029 (54%) with no-mild MR at baseline. The validation cohort included 522 patients (33%) with moderate-to-severe MR and 1076 (66%) with no-mild MR at baseline. Compared to patients with no-mild MR, those with moderate-to-severe MR had lower body mass index, higher comorbidity burden, signs and symptoms of more severe HF, lower systolic blood pressure, and larger left atrial and ventricular dimensions, in both cohorts. NT-proBNP, CA125, fibroblast growth factor 23 (FGF23) and growth hormone 1 (GH1) were up-regulated, whereas leptin (LEP) was down-regulated in patients with moderate-severe MR versus no-mild MR, in both index and validation cohorts.

Conclusion: Circulating biomarkers differently expressed in HF patients with moderate-severe MR versus no-mild MR were related to congestion, lipid and mineral metabolism and oxidative stress. These findings may be of value for the development of novel treatment targets in HF patients with MR.

Key words: Heart Failure; Biomarkers; Mitral regurgitation

INTRODUCTION

Analysis of circulating biomarkers is a well-established method to identify pathophysiological pathways in specific heart failure (HF) phenotype and potentially may identify new therapeutic targets. Evidence-based pharmacological treatments for HF (i.e. neurohormonal antagonists) target circulating biomarkers, such as catecholamines, angiotensin, and aldosterone, which were shown to be over expressed in HF patients, and associated with HF severity and progression (1). Furthermore, several studies have shown that patients characteristics (i.e. age) and HF etiology (i.e. ischaemic versus non-ischaemic) significantly influence pathway over- or under-expression, unveiling potentially different and specific biomarkers and therapeutic targets (2,3)

Mitral regurgitation (MR) is a frequent cardiovascular comorbidity in patients with heart HF, is associated with a poor prognosis and has recently been identified as a possible therapeutic target in selected HF patients (4-8). A few studies evaluated circulating plasma proteins in patients with MR by a proteomic approach. They included small cohorts of patients with degenerative mitral valve disease, and used healthy individuals as control (9,10). However, there is no evidence regarding pathophysiological pathways in patients with symptomatic HF and MR.

The aim of this study was to compare differentially expressed circulating biomarkers in HF patients with or without moderate/severe MR included in two independent large cohorts.

METHODS

Patient population

A Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) was a European project, including two prospective, observational, multinational cohorts. The design and

primary results of BIOSTAT-CHF have been published elsewhere (11). Briefly, the index cohort included a total of 2516 patients from eleven centers in Europe, with signs and symptoms of worsening HF and either a left ventricular ejection fraction (LVEF) $\leq 40\%$, or plasma concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) >2000 ng/L. Included patients were either treatment naïve or receiving $<50\%$ of the target dose of angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) and beta-blockers at the time of inclusion. Patients were enrolled from December 2010 to December 2012. Median follow-up was 21 months. A second independent cohort from six centers in Scotland, the validation cohort, included 1738 patients with HF and a previous documented HF admission requiring diuretic treatment, not previously treated or receiving $\leq 50\%$ of target doses of ACEi/ARBs and/or beta-blockers. Patients were recruited from October 2010 until April 2014. Median follow-up was 21 months. This study complies with the Declaration of Helsinki, and medical ethics committees of participating centers approved the study. All patients provided written informed consent.

Mitral regurgitation assessment

Patients underwent two-dimensional transthoracic echocardiography at inclusion using a commercially available echocardiography (3.5 MHz probe). MR was identified and evaluated using two-dimensional and color Doppler echocardiography. According to the study protocol, only the presence of moderate or severe MR at baseline echocardiography (as compared to no or mild MR) was recorded. Both patients with primary and secondary MR were enrolled, but detailed data on MR mechanism or etiology were not collected.

Biomarkers

The Olink Proteomics® Multiplex Cardiovascular (CVD) -II, CVD-III, Immune Response and Oncology-II panels of 363 unique proteins from different pathophysiological domains were used to investigate the biomarker profiles in both BIOSTAT-CHF index and validation cohorts. Complete data about both biomarkers and mitral regurgitation were available in 1917/2516 and 1598/1738

patients, from the index and validation cohorts respectively. NT-proBNP is named natriuretic peptide B (NPPB), cancer antigen (CA125) is named mucin 16 cell surface associated (MUC16).

Statistical analyses

The normal distribution of continuous variables was explored with Kolmogorov-Smirnov and the Shapiro-Wilk tests. Continuous variables following a normal distribution are reported as mean \pm standard deviation (SD) and were compared using the Student t test, whereas those not following a normal distribution are presented as median and interquartile range (IQR) and were compared with the Mann-Whitney U test. Categorical variables are reported as counts and percentages and were compared using the χ^2 or Fisher exact tests, as appropriate.

Differences in expression of biomarkers in HF patients with or without MR were tested using Linear Models for Microarray data analysis (Limma) software (version 3.34.9), using a fold change cut-off of 1.15 (equal to a log₂ fold change cut-off of 0.2), and a false discovery rate <0.05 according to the Benjamini-Hochberg method.

The impact of MR on the effect of the differentially expressed biomarkers on clinical outcome were investigated using a backward stepwise Cox regression. A composite end-point including HF hospitalization and all-cause death was used. Forrest plots and spline curves were obtained. Data were reported as hazard ratio and 95% confidence interval.

All tests were performed two-sided, and a p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using R, A Language and Environment for Statistical Computing, version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria), IBM SPSS Statistics for Windows, version 23.0.0.3 (Armonk, NY: IBM Corp.), and Cytoscape (version 3.8.0) with plugin ClueGO (version 2.5.7) and CluePedia (version 1.5.7) 24, 25.

RESULTS

Baseline characteristics

Index cohort

Out of the 1917 patients of the index cohort included in this analysis 888 (46%) were affected by moderate-to-severe MR, while 1029 (54%) had no/mild MR at baseline. Compared to patients with no/mild MR, those with moderate-to-severe MR had lower body mass index and were less likely to have a history of previous cardiac surgery for valvular heart disease. They were also more likely to have chronic kidney disease, advanced symptoms and signs of heart failure (higher New York Heart Association [NYHA] class and hepatomegaly), lower levels of systolic blood pressure and to receive mineralocorticoid receptor antagonist (MRA) and digoxin (**Table 1**). Finally, larger left ventricular and atrial dimensions were observed in patients with moderate-to-severe MR compared to those with no-mild MR (**Table 2**).

Validation cohort

Out of the 1598 patients of the validation cohort included in this analysis 522 (33%) were affected by moderate-to-severe MR, while 1076 (66%) had no/mild MR at baseline. Compared to patients with no/mild MR, those with moderate-to-severe MR were less likely to be males, and, as in the index cohort, had lower body mass index (BMI). They also had a lower prevalence of ischaemic HF etiology and peripheral arterial disease, whereas atrial fibrillation and chronic kidney disease were more common in this group compared to that including patients with no/mild MR. Moreover, they had lower systolic blood pressure levels and were more likely to receive beta-blockers, MRA and digoxin (**Table 3**). Finally, echocardiographic features distinguishing the two groups in the index cohort were similar to the validation cohort (**Table 4**).

Biomarker profiles

Index cohort

In the index cohort 5 proteins (fibroblast growth factor 21 [FGF21], fibroblast growth factor 23 [FGF23], growth hormone 1 [GH1], MUC16, and NPPB) were up-regulated, whereas 2 proteins were down-regulated (leptin [LEP] and acid phosphatase 5 tartrate resistant [ACP5]) in patients with moderate-to-severe MR compared to those with no/mild MR (**Figures 1 and 2**). Biomarkers' expression in the index cohort stratified by left ventricular ejection fraction (> or = versus <50%) and history of atrial fibrillation is reported in **Supplementary Figure 1**.

Among these biomarkers, 3/5 of up-regulated (FGF23, MUC16 and NPPB) and 1/2 of down-regulated (LEP) were significantly correlated with hospitalization for heart failure and/or death, as displayed in **Supplementary Figure 2**. The impact of these biomarkers on outcome was not affected by MR status (**Supplementary Table 1**).

Validation cohort

In the validation cohort 9 proteins were up-regulated in patients with moderate-to-severe MR, with 4 (FGF23, NPPB, GH1 and MUC16) overlapping with the 5 biomarkers found to be up-regulated in the index cohort. On the other hand, LEP was the only down-regulated biomarker in patients with moderate-to-severe versus no-mild MR (**Figure 1 and 3**). Biomarkers' expression in the validation cohort stratified by left ventricular ejection fraction (> or = versus <50%) and history of atrial fibrillation is reported in **Supplementary Figure 1**.

FGF23, MUC16 and NPPB over-regulation was significantly associated with clinical outcome in the validation cohort, coherently with results of the index cohort, whereas LEP did not show significant prognostic value (**Supplementary Figure 3**).

There was a significant interaction between MR and the impact of ACP5 on hospitalization for HF or all-cause death (**Supplementary Table 2 and Supplementary Figure 4**).

DISCUSSION

Using a panel of biomarkers from different biological domains in two large, independent cohorts of patients with HF, we identified multiple differences in protein expression between patients with moderate-to-severe MR versus those with no or mild MR. In particular, in both the validation and index cohort we found a significant over-expression of NPPB, MUC16, FGF23 and GH1 and under-expression of LEP in patients with moderate-to-severe MR compared to those with no-mild MR.

Markers of congestion and heart failure severity

NPPB, released by cardiomyocytes in response to LV stress, is, together with BNP, the most validated biomarker for HF diagnosis and risk prognostication.¹² Higher NPPB values in patients with HF and moderate-to-severe MR, compared to those with no-mild MR, and their strong association with unfavorable outcome, were already observed in previous studies. Successful correction of MR, when associated with a positive reverse remodeling, leads to a reduction in natriuretic peptides circulating levels (12). High NPPB levels are associated with poor prognosis in HF patients undergoing percutaneous treatment of secondary MR (13).

MUC16, also called serum carbohydrate antigen 125 (Ca125), is produced by serous epithelial cells and mesothelial cells as a response to congestion and inflammation, and elevated circulating levels has been associated with adverse outcome in heart failure (14). Previous analyses have investigated the predictive role MUC16 in the BIOSAT-CHF index cohort, showing, consistently with our results, a strong association with adverse prognosis and NPPB levels (15). High levels of MUC16 were observed in patients with functional MR in a small study including 77 patients with non-ischaeamic dilated cardiomyopathy (16). Over-expression of this protein in patients with significant MR was confirmed in our larger population.

FGF23 is a phosphaturic hormone primarily secreted by osteocytes to maintain phosphate and mineral homeostasis. In patients with chronic kidney disease elevation of circulating FGF23 has been linked with cardiac remodeling leading to left ventricle hypertrophy, with strong correlation

with increased with HF events and cardiovascular mortality (17,18). Subsequent studies have shown that FGF23 over expression is common and predicts severe prognosis in patients with acute and chronic HF even without renal dysfunction (33,34). In the BIOSTAT-CHF index cohort higher plasma FGF23 levels were independently associated with volume overload, less successful up-titration of ACE inhibitors (or angiotensin receptor blockers) and an increased risk of all-cause mortality and HF hospitalization (21).

Altogether these findings appear to be in line with current evidence that considers NPPB, MUC16 and FGF23 as reliable biomarkers of HF severity with a strong relation to congestion status, and further stress the role of MR in the pathological cascade of HF.

FGF23 and mitral annular calcification

FGF23 has been proposed as a potential biomarker for vascular and cardiac calcifications, coherently with its role in mineral metabolism. Silva et al. have demonstrated that high FGF23 levels were independent predictors of mitral annular calcification (MAC) in a study population of 150 diabetic patients with chronic kidney disease (22). In 3585 patients included in the Cardiovascular Health Study FGF23 and fetuin-A were found to be significantly associated with higher MAC rates (23). Serum FGF23 levels were associated to accelerated annual MAC progression, assessed by repeated computed tomography, in 6547 patients enrolled in the Multi-Ethnic Study of Atherosclerosis study (24).

As our echocardiographic data did not include MAC, we were not able to test its association with MR severity or FGF23 levels. Nevertheless, the over expression of FGF23 in patients with moderate-to-severe MR may be partly explained by the possible presence of MAC in a population with high rates of chronic kidney disease. As MAC is emerging as a negative prognostic factor in MR, especially when associated with trans-mitral pressure gradient (25), and also represents a limit to both surgical and transcatheter correction approaches (26), further research to clarify the pathway leading to its development are needed.

Dysregulation of growth hormone cascade

GH1, secreted by the pituitary gland, stimulates through its specific receptors the activation of a complex signaling cascade that leads to hepatic production of IGF1. IGF1 is an anabolic hormone, pivotal for increasing bone length and density and muscle mass during growth. IGF1 acts on the cardiovascular system in different ways. It enhances the production of nitric oxide reducing systemic vascular resistance, induces the reuptake of calcium by the sarcoplasmic reticulum by regulating sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA2) and attenuates left ventricular remodeling in experimental models of HF (27).

Patients with cachexia and advanced HF presented increased GH1 but lower IGF1 levels compared with non-cachectic individuals. As the IGF-1/GH ratio in non-cachectic was 12-fold higher in non-cachectic patients than in cachectic patients, GH resistance (defined as high GH levels and low IGF-1 levels) is thought to be associated with advanced HF (28). We could speculate that a dysregulation of GH axis may induce oxidative stress, left ventricular remodeling and cardiac cachexia.

The over expression of GH in patients with significant MR in our cohort is coherent with their status of “sicker” patients with advanced HF.

Leptin and cachexia in heart failure

Leptin is a cell-signaling molecule associated with central obesity and visceral fat; it is released by adipocytes, especially by visceral fat, and acts on the hypothalamus to promote satiety, reduce food intake and increase energy expenditure. Its peripheral effects are thought to be pivotal in obesity associated HF. Maladaptive effects of hyperleptinemia in obesity encompass stimulation of sympathetic nervous and renin-angiotensin systems, enhancing aldosterone secretion, sodium retention and heart remodeling (29). In HF patients circulating leptin levels are markedly elevated and associated with adverse prognosis (30). However, studies have shown that plasma leptin

concentration are low in HF patient with cachexia compared to non-cachectic patient, even after correction for body fat content (31,32).

In our study leptin was under expressed in patients with moderate-to-severe MR both in the index and validation cohort. Cachexia incidence in our study populations was not available, but BMI was significantly lower in patients with MR compared to those without in both cohorts. As patients with MR had more advanced HF, a higher incidence of cachexia in this group was likely, and may account for leptin under expression.

LIMITATIONS

We have to recognize several limitations of the present study. Firstly, the results of this study were based on a post-hoc analysis. Moreover, echocardiographic images at baseline were not evaluated by a Core Laboratory and echocardiographic data, including MR, are site-reported without specific protocol or information regarding loading conditions provided. Notably, details about MR degree (i.e. differentiation between none versus mild versus moderate versus severe MR) and etiology (i.e. differentiation between primary and secondary MR) are not available. Finally, populations included are not contemporary heart failure populations since enrolment period was 2010-2014. Nevertheless, our findings will likely inform future studies aimed at better evaluate diagnostic and therapeutic tools in patients with MR and HF.

CONCLUSIONS

In two large independent HF populations, biomarkers associated with congestion, HF severity (i.e. NPPB, MUC16, FGF23) and mineral metabolism (i.e. FGF23), were up-regulated in patients with moderate-to-severe MR compared to those with no-mild MR. GH1 over-expression, associated with oxidative stress and left ventricular remodeling, and LEP under-expression, associated with lipid

metabolism and cachexia, were also observed in patients with moderate-to-severe MR versus no-mild MR. These findings provide new insights regarding possible new therapeutic targets in patients with HF and MR.

REFERENCES

- 1) Smith JG. Molecular Epidemiology of Heart Failure: Translational Challenges and Opportunities. *JACC Basic to Transl Sci.* 2017;2(6):757-769.
- 2) Sama IE, Woolley RJ, Nauta JF, et al. A network analysis to identify pathophysiological pathways distinguishing ischaemic from non-ischaemic heart failure. *Eur J Heart Fail.* 2020;22(5):821-833.
- 3) Ferreira JP, Ouwerkerk W, Santema BT, et al. Differences in biomarkers and molecular pathways according to age for patients with HFrEF. *Cardiovasc Res.* 2021;117(10):2228-2236.
- 4) Pagnesi M, Adamo M, Sama IE, ET AL. Impact of mitral regurgitation in patients with worsening heart failure: insights from BIOSTAT-CHF. *Eur J Heart Fail.* 2021 Oct;23(10):1750-1758.
- 5) Pagnesi M, Adamo M, Sama IE, ET AL. Clinical impact of changes in mitral regurgitation severity after medical therapy optimization in heart failure. *Clin Res Cardiol.* 2022 Mar 16. doi: 10.1007/s00392-022-01991-7.
- 6) McDonagh TA, Metra M, Adamo M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). With the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail.* 2022;24(1):4-131.
- 7) Giannini C, D'ascenzo F, Fiorelli F, et al. A meta-analysis of MitraClip combined with medical therapy vs. medical therapy alone for treatment of mitral regurgitation in heart failure patients. *ESC Heart Fail.* 2018;5(6):1150-1158.
- 8) Adamo M, Fiorelli F, Melica B, et al. COAPT-Like Profile Predicts Long-Term Outcomes in Patients With Secondary Mitral Regurgitation Undergoing MitraClip Implantation. *JACC Cardiovasc Interv.* 2021;14(1):15-25.
- 9) Tan HT, Ling LH, Dolor-Torres MC, Yip JWL, Richards AM, Chung MCM. Proteomics discovery of biomarkers for mitral regurgitation caused by mitral valve prolapse. *J Proteomics.* 2013;94:337-345.
- 10) Deroyer C, Magne J, Moonen M, et al. New biomarkers for primary mitral regurgitation. *Clin Proteomics.* 2015;12(1):1-14.
- 11) Voors AA, Anker SD, Cleland JG, et al. A systems BIOlogy Study to TAIlored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail.* 2016;18(6):716-726.

- 12) Kainuma S, Taniguchi K, Toda K, et al. B-type natriuretic peptide response and reverse left ventricular remodeling after surgical correction of functional mitral regurgitation in patients with advanced cardiomyopathy. *J Cardiol.* 2015;66(4):279-285.
- 13) Godino C, Scotti A, Taramasso M, et al. Two-year cardiac mortality after MitraClip treatment of functional mitral regurgitation in ischemic and non-ischemic dilated cardiomyopathy. *Int J Cardiol.* 2018;269:33-39.
- 14) Núñez J, de la Espriella R, Miñana G, et al. Antigen carbohydrate 125 as a biomarker in heart failure: a narrative review. *Eur J Heart Fail.* 2021;23(9):1445-1457.
- 15) Shi C, van der Wal HH, Silljé HHW, et al. Tumour biomarkers: association with heart failure outcomes. *J Intern Med.* 2020;288(2):207-218.
- 16) Karaca O, Guler GB, Guler E, et al. Serum Carbohydrate Antigen 125 Levels in Nonischemic Dilated Cardiomyopathy: A Useful Biomarker for Prognosis and Functional Mitral Regurgitation. *Congest Heart Fail.* 2012;18(3):144-150.
- 17) Isakova T, Xie H, Yang W, et al. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. *JAMA.* 2011;305(23):2432-2439.
- 18) Gutiérrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med.* 2008;359(6):584-592.
- 19) Kestenbaum B, Sachs MC, Hoofnagle AN, et al. Fibroblast growth factor-23 and cardiovascular disease in the general population: the Multi-Ethnic Study of Atherosclerosis. *Circ Heart Fail.* 2014;7(3):409-417.
- 20) Seiler S, Cremers B, Rebling NM, et al. The phosphatonin fibroblast growth factor 23 links calcium-phosphate metabolism with left-ventricular dysfunction and atrial fibrillation. *Eur Heart J.* 2011;32(21):2688-2696.
- 21) ter Maaten JM, Voors AA, Damman K, et al. Fibroblast growth factor 23 is related to profiles indicating volume overload, poor therapy optimization and prognosis in patients with new-onset and worsening heart failure. *Int J Cardiol.* 2018;253:84-90.
- 22) Silva AP, Gundlach K, Büchel J, et al. Low magnesium levels and FGF-23 dysregulation predict mitral valve calcification as well as intima media thickness in predialysis diabetic patients. *Int J Endocrinol.* 2015;2015.
- 23) Bortnick AE, Bartz TM, Ix JH, et al. Association of inflammatory, lipid and mineral markers with cardiac calcification in older adults. *Heart.* 2016;102(22):1826-1834.
- 24) Bortnick AE, Xu S, Kim RS, et al. Biomarkers of mineral metabolism and progression of aortic valve and mitral annular calcification: The Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis.* 2019;285:79-86.
- 25) Bertrand PB, Churchill TW, Yucel E, et al. Prognostic importance of the transmitral pressure gradient in mitral annular calcification with associated mitral valve dysfunction. *Eur Heart J.* 2020;41(45):4321-4328.

- 26) Abramowitz Y, Jilaihawi H, Chakravarty T, Mack MJ, Makkar RR. Mitral Annulus Calcification. *J Am Coll Cardiol*. 2015;66(17):1934-41.
- 27) Marra AM, Bobbio E, D'Assante R, et al. Growth Hormone as Biomarker in Heart Failure. *Heart Fail Clin*. 2018;14(1):65-74.
- 28) Anker SD, Volterrani M, Pflaum CD, et al. Acquired growth hormone resistance in patients with chronic heart failure: Implications for therapy with growth hormone. *J Am Coll Cardiol*. 2001;38(2):443-452.
- 29) Packer M. Leptin-Aldosterone-Nephrilysin Axis: Identification of Its Distinctive Role in the Pathogenesis of the Three Phenotypes of Heart Failure in People with Obesity. *Circulation*. 2018;137(15):1614-1631.
- 30) Bobbert P, Jenke A, Bobbert T, et al. High leptin and resistin expression in chronic heart failure: Adverse outcome in patients with dilated and inflammatory cardiomyopathy. *Eur J Heart Fail*. 2012;14(11):1265-1275.
- 31) Murdoch DR, Rooney E, Dargie HJ, Shapiro D, Morton JJ, McMurray JJV. Inappropriately low plasma leptin concentration in the cachexia associated with chronic heart failure. *Heart*. 1999;82(3):352-356.
- 32) Filippatos GS, Tsilias K, Venetsanou K, et al. Leptin serum levels in cachectic heart failure patient. Relationship with tumor necrosis factor- α system. *Int J Cardiol*. 2000;76(2-3):117-122.

FIGURE LEGENDS

Figure 1. Venn diagram of number of differentially expressed proteins in patients with moderate-severe mitral regurgitation (MR) versus no-mild MR in index cohort and validation cohort.

Figure 2. Volcano plots of differential protein expression in patients with moderate-severe MR versus no-mild MR in the index cohort

Figure 3. Volcano plots of differential protein expression in patients with moderate-severe MR versus no-mild MR in the validation cohort

Table 1 Baseline clinical characteristics of the index cohort stratified by presence of moderate to severe mitral regurgitation

	Overall (n = 1917)	Moderate or severe MR (n = 888)	No or mild MR (n = 1029)	P- value
Age (years)	68.8 ± 12.1	68.6 ± 11.8	68.9 ± 12.3	0.538
Male sex	1411 (73.6)	656 (73.9)	755 (73.4)	0.804
BMI (kg/m ²)	27.8 ± 5.4	27.3 ± 5.2	28.1 ± 5.6	0.001
Race				0.126
Caucasian	1894 (98.8)	877 (98.8)	1017 (98.8)	
Asian	10 (0.5)	3 (0.3)	7 (0.7)	
Black	4 (0.2)	4 (0.4)	0 (0)	
Other	9 (0.5)	4 (0.4)	5 (0.49)	
Previous HF hospitalization(s) in last year	589 (30.7)	280 (31.5)	309 (30.3)	0.477
Primary ischaemic HF aetiology	844 (44.9)	395 (45.4)	449 (44.5)	0.73
Smoking				0.203
Past	919 (48)	420 (47.5)	499 (48.5)	
Current	283 (14.8)	120 (13.6)	163 (15.8)	
Medical history				
Hypertension	1183 (61.7)	538 (60.6)	645 (62.7)	0.346
Diabetes	606 (31.6)	273 (30.7)	333 (32.4)	0.447
Myocardial infarction	707 (36.9)	315 (35.5)	392 (38.1)	0.235
CABG	327 (17.1)	147 (16.5)	180 (17.5)	0.586
Previous valvular surgery	138 (7.2)	51 (5.7)	87 (8.45)	0.022
Previous PCI	388 (20.2)	163 (18.4)	225 (21.9)	0.056
Atrial fibrillation	870 (45.4)	407 (45.8)	463 (45)	0.713
Stroke	183 (9.5)	84 (9.5)	99 (9.6)	0.904
Peripheral arterial disease	205 (10.7)	93 (10.5)	112 (10.9)	0.771
Chronic kidney disease	539 (28.1)	274 (30.9)	265 (25.7)	0.013
Current malignancy	71 (3.7)	30 (3.4)	41 (4)	0.484
Chronic obstructive pulmonary disease	328 (17.1)	144 (16.2)	184 (17.9)	0.334
Device therapy				0.31
Pacemaker	138 (7.2)	59 (6.6)	79 (7.7)	
ICD	134 (7)	69 (7.8)	65 (6.3)	
CRT-P	33 (1.7)	18 (2)	15 (1.5)	
CRT-D	118 (6.1)	58 (6.5)	60 (5.8)	
NYHA functional class				0.002
I	40 (2.1)	18 (2.1)	22 (2.2)	

II	690 (37)	291 (33.5)	399 (40.1)	
III	902 (48.4)	429 (49.4)	473 (47.5)	
IV	231 (12.4)	130 (15)	101 (10.1)	
Clinical profile				
Peripheral oedema	458 (28.9)	226 (30)	232(27.8)	0.335
Hepatomegaly	263 (13.8)	153 (17.3)	110 (10.7)	< 0.001
SBP (mmHg)	125 ± 22	123 ± 22	126 ± 23	< 0.001
DBP (mmHg)	75 ± 13	75 ± 13	75 ± 14	0.329
HR (bpm)	80 ± 19	81 ± 19	79 ± 20	0.068
Type of visit				0.059
Inpatient hospitalization	1356 (70.7)	642 (72.3)	714 (69.4)	
Outpatient (scheduled)	439 (25)	202 (22.7)	277 (26.9)	
Outpatient (unscheduled)	82 (4.3)	44 (4.9)	38 (3.7)	
HF therapy				
ACE/ARB use	1388 (72.4)	627 (70.6)	761 (74)	0.102
B-blocker use	1597 (83.3)	731 (82.3)	866 (84.1)	0.281
MRA use	1007 (52.5)	511 (57.5)	496 (48.2)	< 0.001
Loop diuretic use	1907 (99.5)	884 (99.5)	1023 (99.4)	0.688
Digoxin use	358 (18.7)	195 (22)	163 (15.8)	0.001

Data are presented as n (%) and mean ± standard deviation.

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass graft; CRT-D, cardiac resynchronization therapy with defibrillator; CRT-P, cardiac resynchronization therapy with pacemaker; DBP, diastolic blood pressure; HF, heart failure; HR, heart rate; ICD, implantable cardioverter-defibrillator; MR, mitral regurgitation; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; SBP, systolic blood pressure.

Table 2: Baseline echocardiographic data and laboratory characteristics of the index cohort stratified by presence of moderate to severe mitral regurgitation.

	Overall (n = 1917)	Moderate or severe MR (n = 888)	No or mild MR (n = 1029)	P-value
Echocardiographic data				
LVEF (%)	30 (25-36)	30 (24-35)	30 (25-38)	< 0.001
LVEF categories				< 0.001
HF _r EF (LVEF < 40%)	1440 (80.8)	718 (84.8)	722 (77.1)	
HF _{mr} EF (LVEF 40-49%)	226 (12.7)	84 (9.9)	142 (15.2)	
HF _p EF (LVEF ≥50%)	117 (6.6)	45 (5.3)	72 (7.7)	
LV remodelling				< 0.001
Normal geometry	285 (21.1)	121 (18.2)	164 (24)	
Concentric remodelling	47 (3.5)	17 (2.6)	30 (4.4)	
Concentric hypertrophy	202 (15)	75 (11.3)	127 (18.5)	
Eccentric hypertrophy	816 (60.4)	452 (68)	364 (53.1)	
LVEDD (mm)	61 (55-67)	63 (57-69)	59 (53-65)	< 0.001
LVESD (mm)	50 (43-57)	52 (45-59)	48 (41-55)	< 0.001
Left atrium diameter (mm)	47 (43-52)	49 (44-54)	46 (41-50)	< 0.001
Laboratory data				
Haemoglobin (g/dL)	13.3 (11.9-14.5)	13.3 (11.9-14.5)	13.3 (11.9-14.5)	0.852
Creatinine (μmol/L)	102 (84-131)	106 (85-132)	100 (84-126)	0.024
eGFR CKD-EPI (mL/min/1.73 m ²)	59 (44-77)	59 (43-76)	60 (45-79)	0.05
Urea (mmol/L)	11 (7.4-17.8)	11.5 (7.7-19.4)	10.5 (7.3-16.4)	0.002
Sodium (mmol/L)	140 (137-142)	140 (137-142)	140 (137-142)	0.645
NT-proBNP (ng/L)	2677 (1204-5587)	3088 (1400-6573)	2433 (1051-5014)	< 0.001

Data are presented as n (%) and median (Q25–Q75).

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HF_{mr}EF, heart failure with mid-range ejection fraction; HF_pEF, heart failure with preserved ejection fraction; HF_rEF, heart failure with reduced ejection fraction; LV, left ventricle; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; MR, mitral regurgitation; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Table 3. Baseline clinical characteristics of the validation cohort stratified by presence of moderate to severe mitral regurgitation

	Overall (n = 1598)	Moderate or severe MR (n = 522)	No or mild MR (n = 1076)	P-value
Age (years)	73.8 ± 10.6	74.5 ± 10.9	73.4 ± 10.5	0.056
Male sex	1063 (66.5)	315 (60.3)	748 (69.5)	< 0.001
BMI (kg/m ²)	28.9 ± 6.3	27.5 ± 6.1	29.6 ± 6.3	< 0.001
Race				0.155
Caucasian	1589 (99.4)	520 (99.6)	1069 (99.3)	
Asian	6 (0.4)	0 (0)	6 (0.6)	
Black	1 (0.06)	1 (0.2)	0 (0)	
Other	2 (0.13)	1 (0.2)	1 (0.1)	
Previous HF hospitalization(s) in last year	423 (26.9)	147 (28.6)	276 (26)	0.282
Primary ischaemic HF aetiology	1035 (93.8)	323 (91)	712 (95.2)	0.002
Smoking				0.048
Past	552 (34.7)	167 (32.2)	385 (36)	
Current	215 (13.5)	85 (16.4)	130 (12.1)	
Medical history				
Hypertension	932 (58.5)	288 (55.6)	644 (59.9)	0.102
Diabetes	518 (32.6)	150 (28.8)	368 (34.4)	0.024
Myocardial infarction	783 (49.1)	241 (46.3)	542 (50.5)	0.115
CABG	288 (18)	87 (16.7)	201 (18.7)	0.322
Previous valvular surgery	102 (6.4)	25 (4.8)	77 (7.2)	0.069
Previous PCI	293 (18.5)	83 (16)	210 (19.8)	0.068
Atrial fibrillation	713 (45)	272 (52.5)	441 (41.3)	< 0.001
Stroke	293 (18.5)	95 (18.4)	198 (18.6)	0.938
Peripheral arterial disease	354 (22.7)	99 (19.4)	255 (24.4)	0.026
Chronic kidney disease	722 (45.7)	272 (52.4)	450 (42.5)	< 0.001
Current malignancy	68 (4.3)	18 (3.5)	50 (4.7)	0.267
Chronic obstructive pulmonary disease	294 (18.5)	85 (16.5)	209 (19.6)	0.14
Device therapy				< 0.001
Pacemaker	107 (6.7)	32 (6.1)	75 (7)	
ICD	64 (4)	34 (6.5)	30 (2.8)	
CRT-P	23 (1.4)	8 (1.5)	15 (1.4)	
CRT-D	49 (3.1)	27 (5.2)	22 (2)	
NYHA functional class				0.697

I	16 (1)	3 (0.57)	13 (1.2)	
II	663 (41.5)	217 (41.6)	446 (41.5)	
III	707 (44.3)	233 (44.6)	474 (44.1)	
IV	211 (13.2)	69 (13.2)	142 (13.2)	
Clinical profile				
Peripheral oedema	431 (30.1)	151 (32.4)	280 (28.9)	0.179
Haepatomegaly	57 (3.9)	19 (4)	38 (3.9)	0.872
SBP (mmHg)	126 ± 22	124 ± 22	127 ± 23	0.004
DBP (mmHg)	69 ± 13	69 ± 12	69 ± 13	0.851
HR (bpm)	74 ± 16	75 ± 17	74 ± 16	0.212
Patient recruited from				
				0.003
Clinic	737 (46.1)	257 (49.2)	480 (44.6)	
General Practitioner	31 (1.9)	2 (0.38)	29 (2.7)	
Ward	830 (51.9)	263 (50.4)	567 (52.7)	
HF therapy				
ACE/ARB use	1127 (70.8)	370 (71.4)	757 (70.6)	0.718
B-blocker use	1155 (72.6)	397 (76.6)	758 (70.6)	0.012
MRA use	516 (32.4)	189 (36.5)	327 (30.5)	0.016
Loop diuretic use	1575 (99)	516 (99.6)	1059 (98.7)	0.085
Digoxin use	288 (18.1)	110 (21.2)	178 (16.6)	0.024

Data are presented as n (%) and mean±standard deviation.

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass graft; CRT-D, cardiac resynchronization therapy with defibrillator; CRT-P, cardiac resynchronization therapy with pacemaker; DBP, diastolic blood pressure; HF, heart failure; HR, heart rate; ICD, implantable cardioverter-defibrillator; MR, mitral regurgitation; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; SBP, systolic blood pressure.

Table 4. Baseline echocardiographic data and laboratory characteristics of the validation cohort stratified by presence of moderate to severe mitral regurgitation.

	Overall (n = 1598)	Moderate or severe MR (n = 522)	No or mild MR (n = 1076)	P-value
Echocardiographic data				
LVEF (%)	40 (35-50)	35 (28-45)	43 (35-52)	< 0.001
LVEF categories				< 0.001
HF _r EF (LVEF < 40%)	702 (46.2)	310 (62.3)	392 (38.1)	
HF _{mr} EF (LVEF 40-49%)	389 (25.6)	106 (21.6)	283 (27.5)	
HF _p EF (LVEF ≥50%)	427 (28.1)	74 (15.1)	353 (34.3)	
LV remodeling				< 0.001
Normal geometry	104 (14.7)	32 (11.8)	72 (16.5)	
Concentric remodeling	83 (11.7)	17 (6.3)	66 (15.1)	
Concentric hypertrophy	242 (34.2)	80 (29.5)	162 (37.2)	
Eccentric hypertrophy	278 (39.3)	142 (52.4)	136 (31.2)	
LVEDD (mm)	55 (48-61)	58 (52-63)	53 (47-59)	< 0.001
LVESD (mm)	44 (36-54)	50 (41-56)	41 (33-49)	< 0.001
Left atrium diameter (mm)	45 (41-50)	47 (44-52)	44 (40-48)	< 0.001
Laboratory data				
Haemoglobin (g/dL)	13.2 (11.8-14.5)	13 (11.8-14.3)	13.3 (11.8-14.7)	0.047
Creatinine (μmol/L)	97 (89-124)	102 (82-133)	95 (79-122)	< 0.001
eGFR CKD-EPI (mL/min/1.73 m ²)	60 (43-78)	56 (39-74)	62 (45-79)	< 0.001
Urea (mmol/L)	8.6 (6.5-12)	9.1 (6.9-13.4)	8.4 (6.4-11.3)	< 0.001
Sodium (mmol/L)	139 (137-141)	139 (137-141)	139 (137-141)	0.985
NT-proBNP (ng/L)	1356 (510-3531)	2270 (900-5319)	1078 (399-2810)	< 0.001

Data are presented as n (%) and median (Q25–Q75).

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HF_{mr}EF, heart failure with mid-range ejection fraction; HF_pEF, heart failure with preserved ejection fraction; HF_rEF, heart failure with reduced ejection fraction; LV, left ventricle; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; MR, mitral regurgitation; NT-proBNP, N-terminal pro-B-type natriuretic peptide

Figure 1.

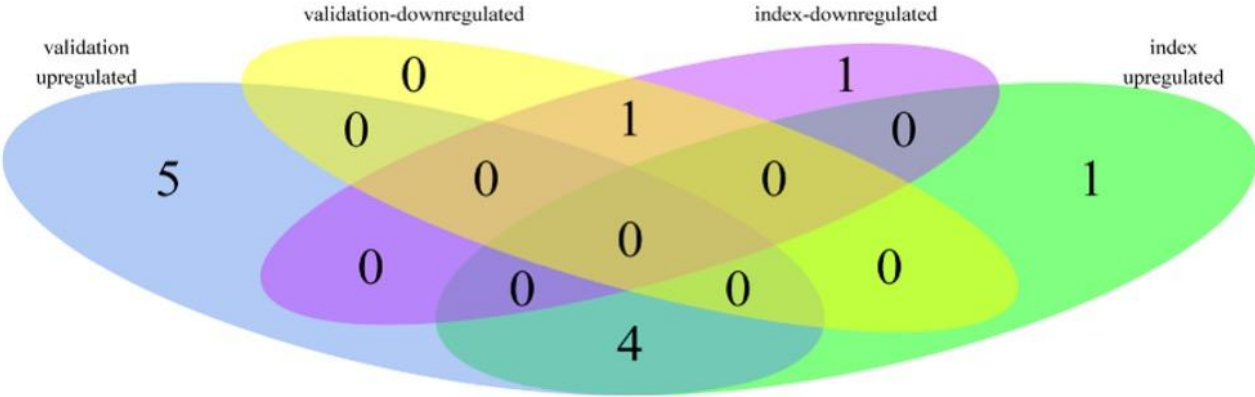


Figure 2.

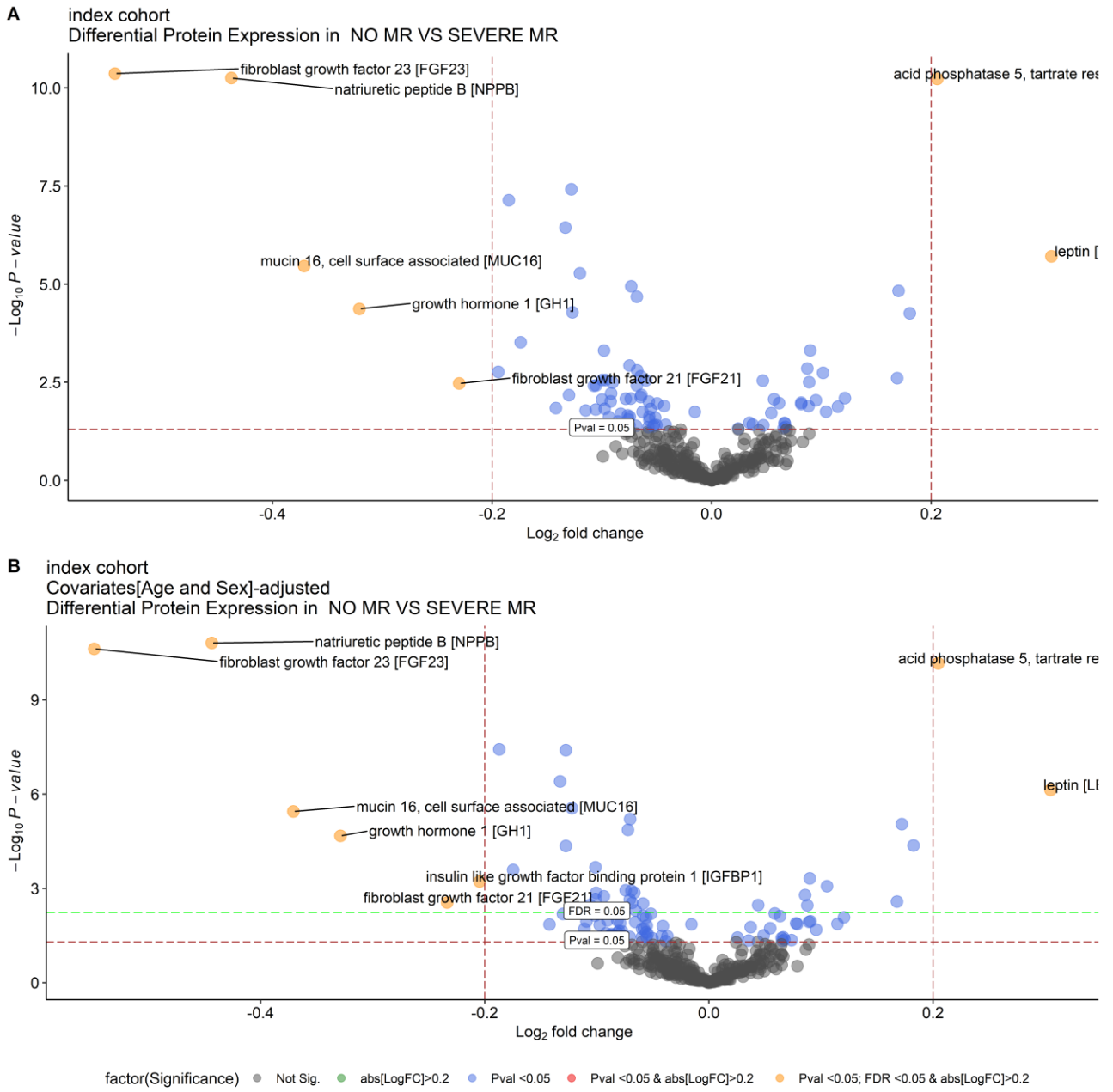


Figure 3.

