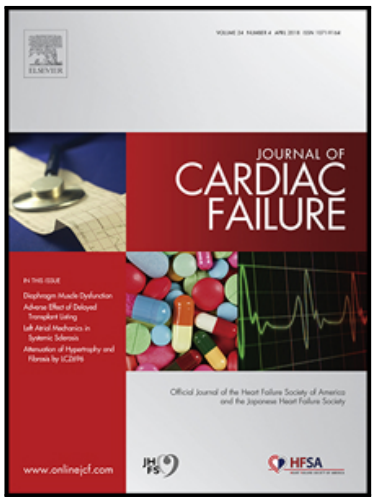

Insulin Growth Factor Phenotypes in Heart Failure with Preserved Ejection Fraction, an INSPIRE Registry and CATHGEN Study

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HIGLIGHTS

- The Insulin Growth Factor (IGF) Axis is a central pathway in HFpEF
- Novel IGF phenotypes were identified centering around IGF-1, IGFBP 1,2 and 7
- Angiopoetin-2 is a potential novel biomarker of pulmonary hypertension in HFpEF
- High dimensional profiling also highlight NT-proBNP, GDF-15, oncostatin M and tissue factor

Insulin Growth Factor Phenotypes in Heart Failure with Preserved Ejection Fraction, an INSPIRE Registry and CATHGEN Study

IGF axis in HFpEF

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ABSTRACT

Background: The insulin like growth factor (IGF) axis emerged as an important pathway in heart failure with preserved ejection (HFpEF). We aimed to identify IGF phenotypes associated with HFpEF in the context high-dimensional proteomic profiling.

Methods: From the Intermountain INSPIRE Registry, we identified 96 patients with HFpEF and matched controls. We performed targeted proteomics including IGF-1,2, IGF binding proteins (IGFBP) 1-7 and 111 other proteins (EMD Millipore and ELISA). We used partial least square discriminant analysis (PLS-DA) to identify a set of proteins associated with prevalent HFpEF, pulmonary hypertension (PH) and 5-year-all-cause mortality. K-mean clustering was used to identify IGF phenotypes.

Results: Patients with HFpEF had a high prevalence of systemic hypertension (95%) and coronary artery disease (74%). Using PLS-DA, we identified a set of biomarkers including IGF1,2 and IGFBP-1,2,7 that provided a strong discrimination of HFPEF, PH and mortality with an AUC of 0.91, 0.77 and 0.83, respectively. Using K mean clustering, we identified three IGF phenotypes that were independently associated with all-cause 5-year mortality after adjustment for age, NT-proBNP and kidney disease (p=0.004). Multivariable analysis validated the prognostic value of IGFBP-1 and 2 in the CATHGEN biorepository.

Conclusion: IGF phenotypes were associated with PH and mortality in HFpEF.

Introduction

Heart failure with preserved ejection fraction (HFpEF) now accounts for at least half the hospitalizations for heart failure.[1] HFpEF is associated with a high burden of comorbidities including coronary artery disease, diabetes mellitus, systemic hypertension and chronic kidney disease as well as chronic pulmonary disease.[1] Survival in patients with HFpEF is closely associated with pulmonary hypertension (PH) [2, 3] . In addition, several biomarkers are predictive of outcome including B-type natriuretic peptide (BNP), ST2 or growth differentiation factor-15 (GDF-15).[2-6]

While HFpEF is a heterogenous syndrome, common mechanisms may contribute to its pathophysiology including impaired cardiac metabolism, inflammation, endothelial dysfunction and neurohormonal activation.[4, 7] In particular, several studies have highlighted the role of insulin as well as the insulin growth factor and binding protein pathway (IGF/IGFBP) in HFpEF.[8-11] IGF binding proteins (IGFBPs) modulate IGF bioavailability and activity by either

binding free IGF (e.g. IGFBP1, IGFBP2) or by blocking IGF interacting receptors (IGFBP7).[12] Previous studies have demonstrated that IGFBPs predict incident heart failure as well as worst survival in patients with HFpEF. [4, 13-16] In particular, IGFBP7 has been associated left ventricular (LV) diastolic dysfunction and PH.[4, 13-16]

To date, however, few studies have evaluated IGF protein axis in the context of high dimensional profiling.[4] Our study had two main objectives: first to determine which IGF/IGFBP proteins are more closely associated with prevalent HFpEF, PH status and 5-year all-cause mortality and second to identify IGF/IGFBP clusters in patients with HFpEF.

METHODS

For this study, we leveraged both Intermountain INSPIRE registry (NCT02450006) and the CATHeterization GENetics (CATHGEN) biorepository (validation cohort). The INSPIRE registry cohort included 96 adult patients with HFpEF and controls without heart failure. Participants were recruited between January 1997 and June 2017 as part of the Intermountain INSPIRE Registry.[17] The **diagnosis of HFpEF** was based on ICD diagnostic codes (ICD-9 code 428.3x, ICD-10 code I50.3x, or ICD-9 codes 428.0, 428.1 or 428.9) after excluding codes related to systolic dysfunction, i.e. ICD-9 codes 428.2 or 428.4 and LVEF < 50%. A B-type natriuretic peptide (BNP) value > 100 pg/mL within one year before or 3 months after the sample draw was also required. Patients **were excluded** if they had hypertrophic cardiomyopathy, amyloidosis, heart transplantation, valvular heart disease, left ventricular assist device, liver cirrhosis, dialysis at time of sample, undergoing treatment for cancer (excluding non-melanoma skin cancer), or had a history of inflammatory disease (including connective tissue disease, ulcerative colitis,

Crohn disease, rheumatoid arthritis). Diagnosis of pulmonary hypertension (PH) relied on echocardiographic-based right ventricular systolic pressure (RVSP) ≥ 40 mmHg (within 1 year of sample draw) and ICD-9 codes 416.8 or ICD-10 codes I27.20, I27.21, I27.22, I27.29 or I29.9. **Controls** did not meet any of the diagnostic codes for heart failure and when available had an LVEF ≥ 50%, BNP <100 pg/mL and same exclusions as the cases. Controls were matched according to age (± 5 years), sex and body mass index. Baseline characteristics including demographics and comorbidities were collected using the Intermountain Enterprise Data Warehouse.

Proteomic profiling

Plasma samples were stored at -80°C and analyzed by Stanford University Human Immune Monitoring Center who were blinded to clinical data. We used targeted immunoassays from EMD Millipore as well as ELISA panels to assess pathways involved in HFpEF (**supplementary table 1**). Among the EMD Millipore panels, we included the IGF and IGFBP panels, the human aging panel-1, the human cardiovascular panels 1 to 4, the human angiogenesis/growth factor panel-1 and a customized 42-plex cytokines panel. In addition, big-endothelin-1, total nitric oxide/nitrate/nitrite and ST2/IL33R levels concentrations were measured with a sandwich enzyme linked immunosorbent (ELISA) assay (R&D) using Infinite® 200 PRO (Tecan, Männedorf, Switzerland). For the EMD immunoassays, we equally distribute samples on plates matching HFpEF and controls according to age, sex, comorbidity and PH status. Plates were read using a Luminex FlexMAP3D instrument.

HFpEF classifiers

Three classifiers were used for proteomic studies**,** e.g. (1) prevalent HFpEF status, (2) pulmonary hypertension status and (3) 5-year all-cause mortality or the combined outcome of 5-year all-

cause mortality or heart failure admission. All-cause death was determined by linkage with Utah Department of Health death certificate data and the social security death master file.

Validation CATHGEN Cohort

A validation sample was selected from the CATHGEN biorepository, which is a sequential biorepository of 9334 patients referred for cardiac catheterization between 2001-2010.[18] We identified 88 subjects with a clinical diagnosis of HFpEF (EF ≥45% with evidence of diastolic dysfunction on echocardiography with grade ≥1) and 88 patients without HF (EF ≥45%, diastolic dysfunction grade=0). Time from cardiac catheterization to all-cause mortality was determined using the Social Security Death Index (SSDI) and National Death Index (NDI). Proteomics profiling was completed using the Olink Target 96 multiplex platform (Olink Proteomics, Uppsala, Sweden). The IGF axis was assessed using different panels, i.e. the development panel (IGF2 receptor: IGF2R), the cardiovascular III panel (IGFBP-1, IGFBP-2, IGFBP-7), the cardiometabolic panel (IGFBP-3, IGFBP-6) and the metabolism panel (insulin like growth factor binding protein like 1, IGFBPL1). IGF-1 and IGF-2 are not available in the Olink panels.

Statistical analysis

INSPIRE registry analysis. Continuous data are presented as median and interquartile range and compared using Mann-Whitney-Wilcoxon test, while categorical data are presented as number and percentage, and compared using the Chi-square or Fisher exact test. Proteomic data preprocessing involved background fluorescence subtraction and plate/batch adjustment (empirical Bayes methodology). The mean fluorescent intensity (MFI) was transformed using the natural logarithm. Analysis included (1) a correlation network of IGF proteins using Spearman rank correlation with rho> 0.3, (2) partial least square discriminant analysis (PLS-DA) of prevalent HFpEF, PH status or outcome and receiver operating characteristics for selected

biomarkers, (3) K-mean clustering of IGF proteins as well as selected proteins, (4) partial correlation maps of proteins emerging on PLS-DA analysis and (5) Cox proportional hazards regression analysis or logistic regression analysis. We selected the PLS-DA model with an optimal number of latent factors predicting the outcome while balancing the risk for under- and overfitting the model using predicted residuals sum of squares. The importance of each biomarker in the outcome prediction was determined from the variable importance in projection (VIP) scores of Wold; biomarkers with a VIP>1.4 were considered influential.[12] Statistical analyses were performed using SPSS® v.19 (SPSS Inc, Chicago, IL), JMP Genomics v.9.0 (SAS Institute Inc., Cary, NC) and R v.3.5.1 (R Core Team, Vienna, Austria) software programs. K-mean clustering was done using Morpheus software (https://software.broadinstitute.org/morpheus).

CATHGEN validation cohort analysis. The Olink platform reports relative protein abundance on a $\log_2$ scale; these values were standardized, so the unit tested here is 1 SD of the log measure of abundance. HFpEF vs. no-HF status was tested for association with individual proteins using logistic regression models, while time to all-cause mortality was tested in HFpEF subjects using Cox proportional hazards models truncated at 5 years after cardiac catheterization, after checking the proportional hazards assumption. Multivariable models in the CATHGEN cohort were adjusted for age, sex, race, BMI, systolic BP, creatinine, and diabetes mellitus status.

RESULTS

INSPIRE registry population

The median age of patients with HFpEF (n=96) was 74 years-old [IQR, 68; 84], with a majority being female (60%). The majority of patients were overweight or obese (77%), and 97% had

systemic hypertension (**Table 1**). There was no significant difference between patients with HFpEF and controls (n=96) with regards to age, sex, ethnicity, body mass index, prevalence of diabetes mellitus and coronary artery disease. Patients with HFpEF had more frequent systemic hypertension, dyslipidemia and history of atrial fibrillation than controls.

The median time between HFpEF diagnosis and blood sample draw was 0.75 years (IQR: 0.04, 3.5). The BNP at blood draw [379.0 pg/mL (IQR: 203.8-676.8)] was not significantly different than the BNP at diagnosis [460.50 pg/mL (IQR:161.50-753.50)] for the naturally transformed values (p=0.93) but lower than maximal BNP [828.0 pg/mL (IQR: 416.3-1433.0)] (p<0.001). This suggests a more compensated state at the time of the blood draw.

Relationship between IGF proteins and selected metabolic proteins

Among IGF proteins, IGFBP-1, 2 and 7 had the highest number of connections with IGBP2 connected to IGBPs 1, 3 and 7 and FABP-3 and IGFBP-7 connected to IGBP-2 and 6, FABP-3 and FGF-21 (**Figure 1**). IGF-1 and 2 were weakly connected to each other with stronger negative associations between IGF-1 and IGFBP5 and IGF-2 and IGFBP-1. In the control group, IGF/IGFBP proteins were associated with age with a stronger relationship found for IGFBP-1 (rho=0.54). In the HFpEF cohort of the INSPIRE registry, renal failure history but not diabetes mellitus was significantly associated with IGFBP-2 (r= 0.25, p=0.016) and IGFBP-7 (r=0.26, p=0.010).

Proteomic profiling of prevalent HFpEF

PLS-DA identified several proteins associated with HFpEF, e.g. NT-proBNP, IGFBP-1, IGFBP-7, angiopoietin 2 (ANG-2), GDF-15, ST2, nitrite, pentraxin 3, oncostatin M, CXCL16, serum amyloid protein (SAP) and tissue factor (**Figure 2A and supplementary Table 2**). The area under the curve (AUC) for discriminating prevalent HFpEF was 0.87 (markers with VIP>1.4), p<0.001 and 0.86, p< 0.001 if NT-proBNP was excluded from the biomarker panel. The individual markers were also significant on ROC analysis with the exception of SAP (**Figure 2B)**. A logistic regression model including NT-proBNP, IGFBP-1 or IGFBP-7, nitrite and angiopoetin-2 had an AUC of 0.81 (0.75, 0.86).

Proteomic profiling and pulmonary hypertension status

Fifty-three (55%) patients with HFpEF had a diagnosis of PH. When compared to patients without PH, patients in the HFpEF-PH had a higher prevalence of diabetes mellitus (49.1% vs. 14.0%, p<0.0001) and a lower hemoglobin (12.4 vs. 13.0 g/dL, p=0.01) **(supplementary Table S3)**. There was also a trend toward older age [76.3 (69.9; 85.3) vs. 72.6 (65.1; 79.2) years, p=0.06] and a higher prevalence of female (67.9% versus 48.8%, p=0.06).

On PLS-DA, the biomarkers associated with PH status included angiopoietin-2 (ANG-2), IGFBP-7, IGF-1 and IGF-2, FGF-21, GDF-15, ICAM-1, Big ET-1, VEGF-D, GCSF, SAP, eotaxin/CCL11 and EGF with a model AUC of 0.77, p<0.0001 (**Figure 3A, B**). Angiopoetin-2 had the highest numerical AUC [0.74 (0.64-0.82)] although not statistically different from the AUC of IGFBP-7 [0.64 (0.53-0.73)]. The set of biomarkers were also associated with estimated RVSP based on echocardiography with ANG-2 having the highest Spearman rank correlation (**Figure 3C and D**).

Proteomic profiling and outcome analysis

Patients with HFpEF were followed for a median of 3.78 [IQR, 1.73; 6.84] years in the INSPIRE registry while controls were followed for 8.66 [IQR, 6.09; 11.54] years. During follow-up, 5-year all-cause mortality was observed in 30 patients with HFpEF (31%) while 58 either died or were readmitted for heart failure (60%). Forty-nine patients (51%) had documented hospitalization for decompensated heart failure at an Intermountain hospital within 5 years of blood sampling. Survival was lower in patients with HFpEF than controls (**Figure 4A**).

On PLS-DA, 5-year all-cause mortality was associated with NT-proBNP, IGF-1, 2/ IGFBP-1 and 2, tissue factor, GDF-15, troponin T, fatty-acid binding protein-3 (FABP3), oncostatin M, epithelial growth factor (EGF) and platelet derived growth factors with a model AUC of 0.83, p< 0.001(**Figure 4B**). NT-proBNP and IGF-1/IGFBP-2 ratio had an AUC of 0.73 and 0.69, p< 0.001, respectively. A multivariable Cox model considering age, sex, renal failure history, NT-proBNP, GDF-15, IGF-1/IGFBP2 ratio, FABP3 and tissue factors retained age, renal failure history, NT-proBNP and the IGF-1/IGFBP2 ratio as independent predictors of outcome with a c-statistic of 0.82 [0.73-0.89]. **Supplementary table S6** and **supplementary Figure 1** summarizes factors retained for 5-year all-cause mortality or rehospitalization for HF.

Integrated IGF phenotypes

Among IGF/IGFBP proteins, IGF-1,2 and IGFBPs 1, 2 and 7 were associated with HFpEF classifiers (F**igure 5A).** Using K-mean clustering, we identified three IGF clusters: **phenotype A** (higher IGF-1, low IGFBPs, n=37); **phenotype B** (intermediate IGF-1, slightly lower IGFBP1,2 but higher IGFBP7, n=25) and **phenotype C** (lower IGF-1, higher IGFBP1 and 2 but variable IGFBP7, n=34) (**Figure 5B**). These phenotypes were associated with survival (**Figure 5C)** and remained significant after multivariable adjustment for age, NT-proBNP and history of renal failure; phenotype C has a HR of 3.91, p=0.004 and phenotype B had a HR of 2.82, p=0.06 when

compared to phenotype A. Building on the concept of bioavailable IGF-1, we explore a novel bioavailable index of IGF-1 integrating the geometric mean of IGFBP-2 and 7 (**Figure 5D**) which tacked the different phenotypes. We selected IGFBP-2 instead of IGFBP-11 since IGFBP-2 is less affected by post-prandial state or aging.[19]

Partial regression diagram and integrated biomarker profile

The biomarkers identified by PLS-DA are not independent of each other (**Figure 6**). Among the IGF/IGFBP proteins, stronger relationships were found between IGF-1 and tissue factor, IGF-2 and vascular endothelial growth-factor-D, IGFBP-1 and GDF-15, IGBP-7 and angiopoetin-2 and h granulocyte colony stimulating factor (inverse relationships). As shown in s**upplementary Figure 2**, using a limited set of biomarkers of key pathways in HFpEF, patients with lower bioavailable IGF-1 usually had higher NT-proBNP, higher GDF-15, higher ANG-2 and higher CRP levels.

CATHGEN validation cohort

The CATHGEN cohort was used to validate signatures associated with IGFBPs. Compared to controls, patients with HFpEF were older (64.7±11.3 vs. 53.1± 12 years, p<0.001), had higher BMI (32.0 ±8.4 vs. 29.2 ±7.7 kg/m^2, p=0.02), a higher prevalence of systemic hypertension (73.9% vs. 53.4%, p= 0.008) and diabetes mellitus (34.1% vs. 12.5%, p=0.001) [**supplementary table S7]**. All the IGFBPs were associated with prevalent HFpEF on univariate analysis with IGFBL1, IGFBP-3 and IGFBP-7 having the strongest evidence on multivariable analysis (**Table 2a**). For all-cause mortality in HFpEF, the strongest adjusted HR were found with IGFBP-1, IGFBP-2, and IGFBP-3 (**Table 2b**).

DISCUSSION

The main finding of our study is that lower bioavailable levels of IGF-1 and 2 is not only associated with prevalent HFpEF but also with PH status and all-cause mortality. In addition, we identified endophenotypes (clusters) of the IGF system that center on IGF-1, IGFBP-1, IGFBP-2 and IGFBP-7. It is important to note that both the INSPIRE and CATHGEN cohorts focus on the subgroup of HFpEF with high burden of co-morbidity including atherosclerotic heart disease and diabetes mellitus.

Sharing structural homology with insulin, insulin-like growth factors such as IGF-1 and 2 play a key role in regulating growth, metabolism as well as involved in disease processes and aging.[13, 20, 21] IGFBPs modulate levels of IGF-1 and 2 by either binding free IGF (e.g. IGFBP-1, IGFBP-2) or by blocking IGF interacting receptors (IGFBP-7).[22] In both the INSPIRE and the CATHGEN registries, higher levels of IGFBP-1 and 2 were associated with all-cause mortality; in addition, lower IGF-1 and 2 were also associated with all-cause mortality in the INSPIRE registry. This is consistent with a large body of literature supporting the prognostic value of lower bioavailable IGF-1.[15, 23, 24] For example, in 300 patients referred for coronary angiography and echocardiography, patients with HFpEF had significantly lower serum IGF-1 than controls free from HF and without echocardiographic signs of LV diastolic dysfunction.[15] Lower levels of IGF-1 have also been shown to increase the risk of atherosclerotic cerebrovascular and coronary disease.[21, 25] Previous studies have also implicated the IGFBPs (especially IGFBP 1, 2 and 7) with incident heart failure and survival. For instance, IGFBP-1 and IGFBP-2 levels independently predicted incident HF and all-cause and cardiovascular mortality in 3523 participants of the Framingham Heart Study.[13] In a retrospective study of 870 patients with established HF (HFpEF and HFrEF), Barataut et al. found that higher IGFBP-2 levels were independently associated with higher cardiovascular mortality during a 1- and 6-year follow-up

period.[14] More recently, several studies have focused on the role of IGFBP-7 in HFpEF. Barroso et al. showed that patients with HFpEF presented higher levels of IGFBP-7 than controls without echocardiographic evidence of LV diastolic dysfunction.[15] Hage et al. showed that IGFBP-7 was associated with the severity of diastolic dysfunction and prognosis in HFpEF.[26] In the RELAX trial (Phosphodiesterase-5 Inhibition to Improve Clinical Status and Exercise Capacity in Heart Failure with Preserved Ejection Fraction), IGFBP-7 level was associated with lower baseline and follow-up peak oxygen consumption (pVO_2) and in addition predicted longitudinal changes in diastolic function and pulmonary pressure. [8, 16] In a more recent study, Sanders-van Wijk et al. showed that IGFBP-7 was a proteomic hub modulating hemodynamic severity and inflammation in HFpEF.[4] In obese-inflammatory based cluster analysis in HFpEF, IGFBP-7 was also shown to significantly vary according to the inflammatory profiles.[11] IGFBP-7 also emerged as a key signaling pathway differentiating HF with reduced versus preserved ejection fraction using 13 000 proteins (Somascan) profiling in the study of Amato et al.[27] Finally, a therapeutic intervention trial showed that neprilysin inhibitors decrease IGFBP-7 levels, highlighting the potential use of the IGFBP-7 axis for trial design.[9] Although IGFBP-7 was associated with HFPEF and PH status in our cohort, it did not emerge as independently associated with mortality in both the INSPIRE and CATHGEN biorepositories. This suggests that integrated IGF axis phenotypes may be more important to consider than individual proteins.

Although lower levels of bioavailable IGF have been consistently associated with worse outcome, the effect of the IGF axis in HFpEF and disease is complex. On one hand, IGF-1 deficiency may promote the development of atherosclerotic cardiovascular disease by impairing the nitric oxide pathway.[21] On the other hand, a decrease in IGF signaling and subsequent phosphoinositide-3-kinase/Protein kinase (PI3K/Akt) activation may shift the metabolic focus

from growth toward maintenance and cellular repair.[21, 28, 29] This can in turn attenuate senescence-related cardiac hypertrophy and interstitial fibrosis, inflammation and oxidative stress which are likely protective in HFpEF.[21] Decreased IGF activation could also decrease tumorogenesis or cancer progression and may explain in part the association between lower circulating IGF and survival in older cohorts.[21]

Importantly, our study highlights the intertwined nature of biomarker profiles in HFpEF. This allowed us to identify simplified IGF endophenotypes that may center on IGF-1 and IGFBP-2 and 7. In addition, IGF proteins were closely linked to other key pathways in HFpEF including proteins involved in metabolism such as FABP3 and FGF-21.[30] IGFBP-7 was also strongly associated with angiopoietin-2, a ligand of the receptor tyrosine kinase Tie-2. ANG-2 is primarily produced by endothelial cells and facilitates angiogenesis (functioning as a vessel-destabilizing molecule and regulating blood vessel maturation).[31] It has been implicated in the pathophysiology of pulmonary arterial hypertension and is differentially expressed in heart failure.[27, 32] In a targeted proteomic study, Chirinos et al. found associations between ANG-2 and heart-failure free survival in patients with HFpEF.[33] Its association with PH status in HFpEF will, however require more studies with careful attention on differentiating its role in versus combined PH. Our study shows a relationship between the IGFBP-7 and fibroblast growth factor-21, an emerging metabolic modulator that has protective effects against hypertrophic insults or pressure overload.[33, 34] IGFBP-1/IGFBP-2 were also closely related to GDF-15, a member of the transforming growth factor-beta superfamily stimulated by inflammation, oxidative stress, and tissue hypoxia or injury and a strong prognostic marker in heart failure.[4, 33] Inflammatory and hemostatic markers were also closely linked to the IGF axis biomarkers. Oncostatin M, which is a pleiotropic cytokine that belongs to the interleukin-6 group of

cytokines, emerged as prognostic in our study. A previous study had reported high levels of oncostatin M in 80 patients with heart failure with reduced ejection fraction,[35] but our study is the first to show higher levels in HFpEF.

One important question is whether these findings can translate in clinical practice. Although lower bioavailable IGF have been consistently associated with worse outcome in HFpEF, routine assessment is unlikely to lead to actionable reclassification of risk. This can be in part explained by the fact that it is also associated with other biomarkers such as NT-proBNP, high sensitivity troponin, ST-2 or GDF-15 which are strongly prognostic of outcome.[2-6, 33] Perhaps, one of the most important contributions of multidimension studies is to identify a set of parsimonious biomarkers capturing key pathways in HFpEF (through feature reduction and outcome studies).[4] For our studies as well as from recent proteomic studies[4, 27, 33], a panel will likely include: NT-proBNP, troponin I or FABP-3, GDF-15, selected IGF biomarkers, FGF-21 or FGF-23, ANG-2, PDGFs, hemostasis markers such as tissue factor or PDGF, focused inflammatory markers and markers of liver, kidney and hematological function. Another important question is whether IGF profiling could lead to targeted therapy. As reduction of the IGF axis appears to be mainly protective, further blocking of GH/IGF-1 pathway could be considered using GH receptor antagonists, somatostatin analogs, and anti-IGF-1R antibodies.[21, 36] These therapies may however have several side effects and the IGF may have many redundant pathways limiting efficacy of a targeted blockage.[21, 36] A more efficient way to target the IGF system will likely be the use of medications with pleotropic effects that also target the IGF pathway. For example, recently sodium-glucose transport protein 2 (SGLT2) inhibitors have been shown to exert part of their effects through inhibition of the insulin/IGF1 pathway.[28]

Our study has several limitations. First, while carefully selected from a large registry, our sample size is small. We were, however, able to validate our findings for IGFBP-1 and 2 in a validation cohort; moreover, our findings validate recent proteomic studies in HFpEF but with greater granularity. Second, the diagnosis of HFpEF and PH status was based on ICD-9 codes. We have, however, used supporting criteria including elevated BNP levels and RVSP. In addition, the majority of patients also had documented rehospitalization for heart failure.

In conclusion, our study identifies IGF phenotypes associated with hemodynamic severity and all-cause mortality in HFpEF. IGF axis biomarkers are closely intertwined with other key pathways in HFpEF.

Three brief bullet points

- Patients with heart failure with preserved ejection fraction have lower levels of bioavailable insulin growth factor (IGF); these lower levels are also with pulmonary hypertension and all-cause mortality.
- The insulin growth factor proteins more strongly associated with HFpEF status and outcome include IGF-1 and 2 as well as IGF binding proteins 1, 2 and 7. These five proteins help identify three distinct IGF phenotypes in patients with HFpEF.
- IGF proteins are closely intertwined with other key proteins in HFpEF including B-type natriuretic peptide, growth/differentiation factor 15, angiopoetin-2 and fatty acid binding proteins.

Lay Summary:

With the aging of our society and the increase in obesity and diabetes, there is a gradual increase in heart failure with preserved ejection fraction (HFpEF). Understanding the different mechanisms involved in HFpEF will not only help identify different subgroups of patients and may help develop targeted therapy. In this study, we demonstrated in two cohorts, that patients with HFpEF have significantly lower levels of bioavailable insulin growth factor (IGF); in addition, lower levels were associated with pulmonary hypertension as well as all-cause mortality. Our study also highlights the intertwined network that links IGF with other important pathways in HFpEF.

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Conflict of interest

No specific conflict of interest specific to this paper with the exception of the research funding received by Actelion Pharmaceuticals.

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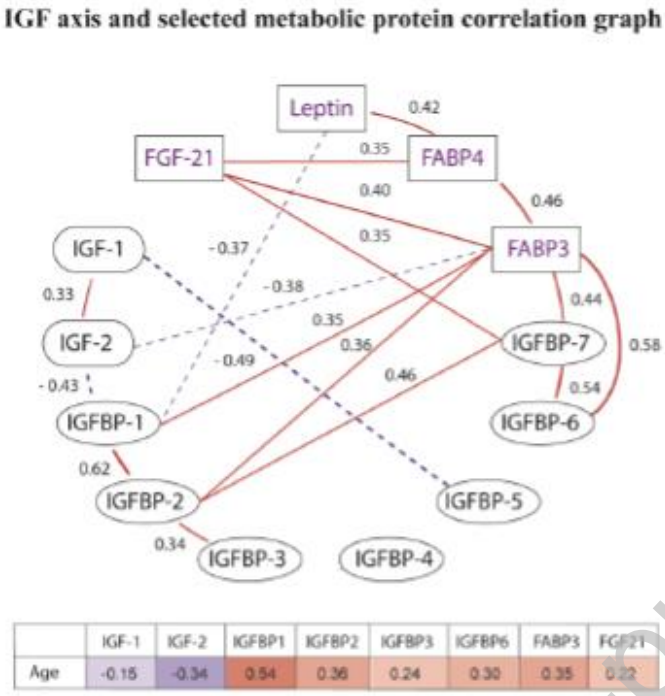

Figure 1. Spearman correlation graph between IGF system proteins and selected metabolomic proteins. Correlation between proteins of the IGF system. Significant correlations with rho >0.3, p< 0.001 are highlighted in both controls and patients with HFpEF. The red lines indicate positive correlation and the blue dashed line negative correlations. The lines are weighted by the strength of the correlations. Among IGF proteins, IGFBP- 2 and 7 had the highest number of connections with IGBP2 connected to IGBP 1, 3 and 7 and the IGFBP7 connected with IGBP2, 6 as with FABP3 and FGF-21. The lower panel shows the association between IGF and metabolic proteins and age in the control group. IGFBP-1 and IGF-2 showed the strongest association with age. presents age related changes with greatest associations found for IGF-2 and IGFBP-1. Abbreviations: IGF indicates insulin growth factor; IGFBP insulin binding protein; FABP, fatty acid binding protein, FGF-21, fibroblast growth factor-21.

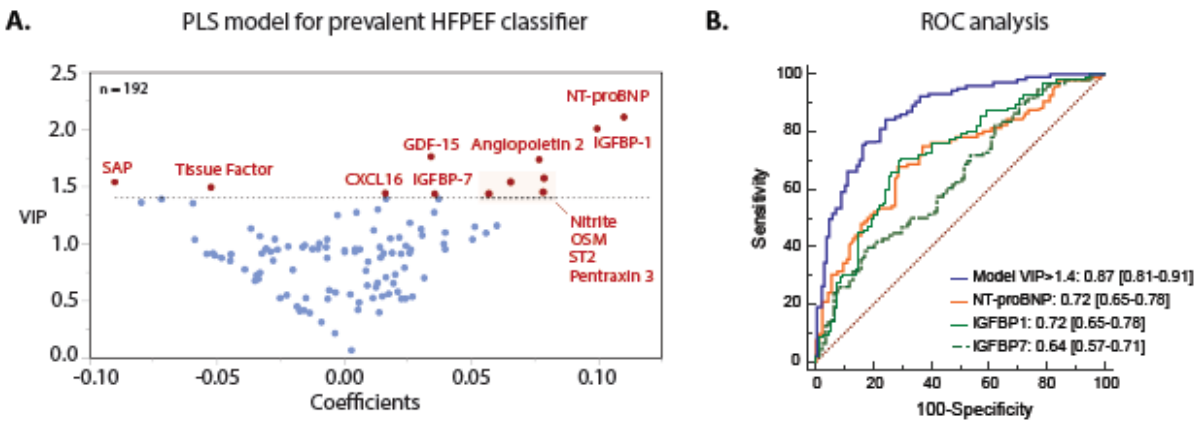

Figure 2. Biomarker Associations with Prevalent Heart Failure with Preserved Ejection Fraction (HFpEF). (A) V-plot was generated from partial least squares discriminant analysis (PLS-DA) for discrimination of HFpEF (n=96) from controls (n=96). Markers with a Variable Importance in Projection (VIP) score above 1.4 were considered influential for discrimination between HFpEF and controls. **(B)** ROC analysis for model discrimination for markers with VIP >1.4, NT-proBNP, IGFBP-1 and IGFBP-2. Abbreviations: IGFBP: insulin growth factor binding protein, GDF, growth differentiating factor; OSM, oncostatin-M; SAP, Serum Amyloid P.

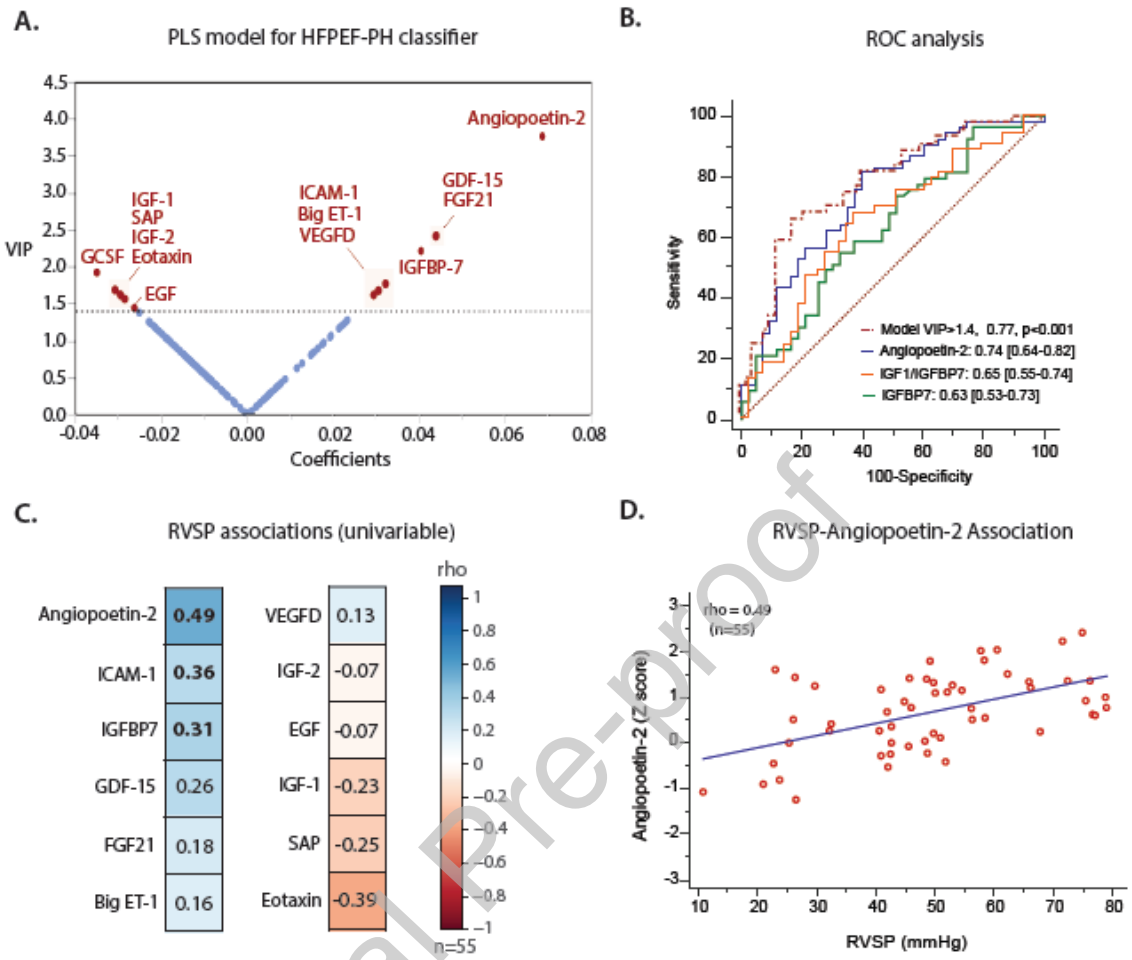

Figure 3. Biomarkers Association with Pulmonary Hypertension status in HFpEF (n=96). **(A)** V-plot was generated from partial least squares discriminant analysis (PLS-DA) for discrimination between HFpEF with and without PH at baseline. Markers with a Variable Importance in Projection (VIP) score above 1.4 were considered influential for discrimination between HFpEF-PH from HFpEF without PH. Correlation coefficients were scaled and centered. **(B)** ROC analysis for model discrimination for markers with VIP >1.4, angiopoetin-2, IGFBP-7 and IGF-1/IGFBP7. **(C)** Spearman correlation coefficients between biomarkers and echocardiographic-estimated right ventricular systolic pressure (RVSP). Correlation coefficients

with p<0.05 are presented in bold. **(D)** Scatterplot between angiopoetin-2 and RVSP. Abbreviations: IGF indicates insulin growth factor; IGFBP insulin binding protein; FABP, fatty acid binding proteins, FGF-21, fibroblast growth factor-21, GDF, growth differentiating factor, ET, endothelin; EGF, epidermal growth factor; GCSF, granulocyte colony stimulating factor; ICAM, soluble intercellular adhesion molecule-1; VEGF, vascular endothelial growth factor; SAP, serum amyloid P.

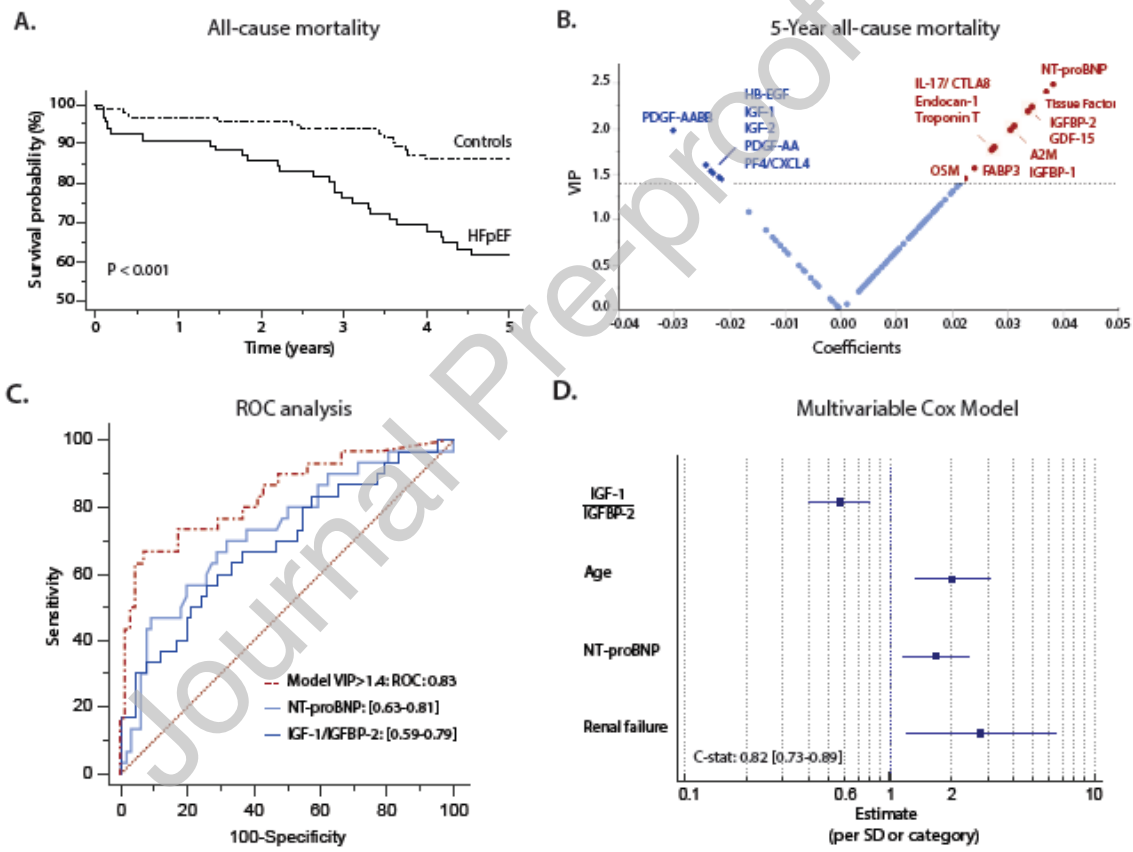

Figure 4. Biomarkers of 5-year all-cause mortality (primary endpoint) (A) Kaplan-Meier survival curve of patients with HFpEF compared to controls. **(B)** Partial least squares analysis of biomarkers predicting outcome in patients with HFpEF. Variable Importance in Projection (VIP)

score above 1.4 were considered influential. Correlation coefficients were scaled and centered. **(C)** ROC analysis for model discrimination for markers with VIP >1.4, angiopoetin-2, NT-proBNP and IGF-1/IGFBP-2. **(D)** Multivariable Cox model demonstrating the independent associations between IGF-1/IGFBP-2, NT-proBNP, age and renal failure history. Abbreviations: IGF indicates insulin growth factor; IGFBP insulin binding protein; GDF, growth differentiating factor; A2M, alpha 2 macroglobulin; FABP, fatty acid binding protein; OSM, oncostatin; HB-EGF; heparin-binding EGF-like growth factor, PDGF, platelet derived growth factor, PF4, platelet factor 4.

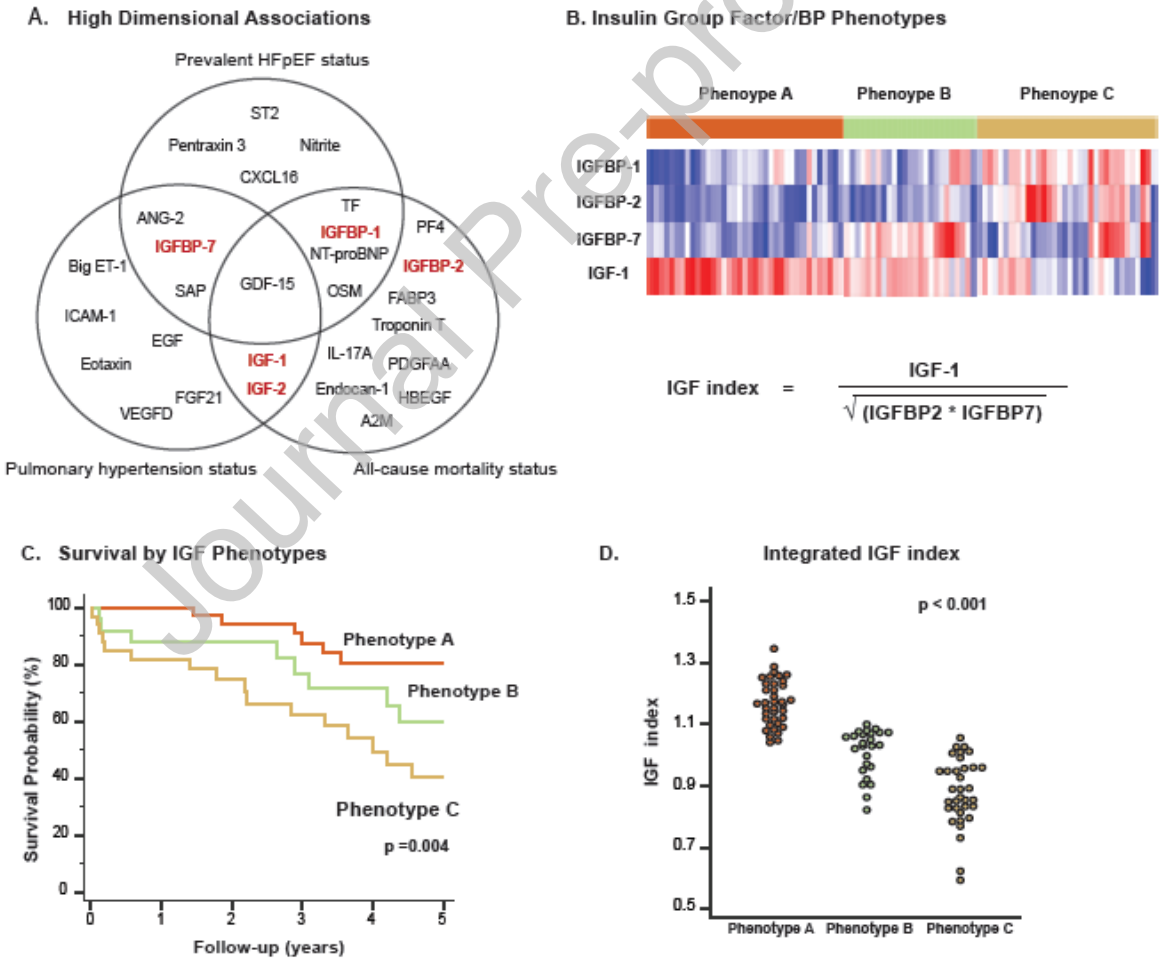

Figure 5. Insulin Growth Factor Phenotypes in Heart Failure and Preserved Ejection Fraction. Section A. Venn diagram of influential factors in PLS-DA for the three classifiers highlighting the central role of the IGF system, GDF-15 and NT-proBNP for discrimination of HFpEF, PH and all-cause mortality risk status. **Section B**. K-mean clustering of selected IGF system phenotypes based on PLS-DA (phenotype A, N=37; phenotype B, N=25, phenotype C, N=34). **Section C**. Kaplan Meir outcome analysis based on IGF phenotypes (phenotype A, N=37; phenotype B, N=25, phenotype C, N=34). **Section D.** Scatterplot comparing the novel bioavailable IGF-1 index across IGF phenotypes (phenotype A, N=37; phenotype B, N=25, phenotype C, N=34). Abbreviations as in previous panels and in supplementary table 1.

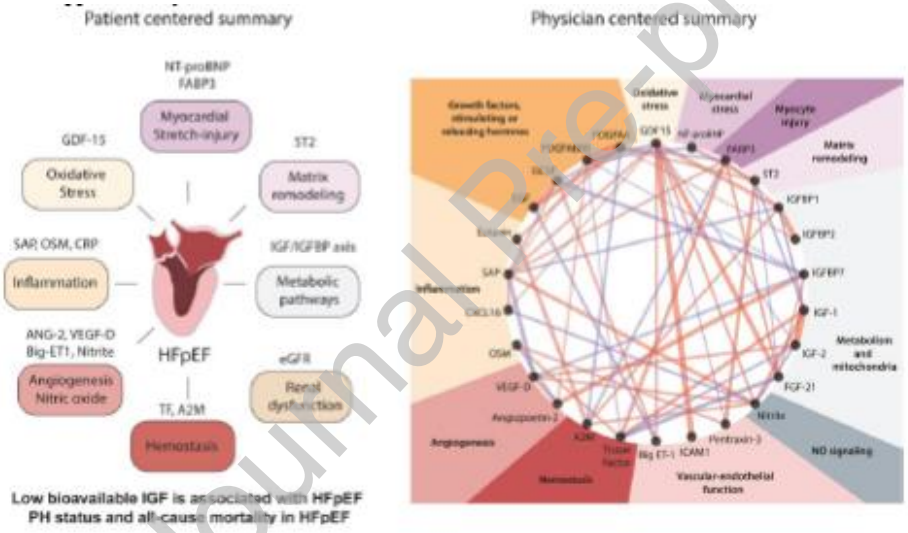

Figure 6. Visual Take Home-The IGF system and multidimensional proteomic profiling. Several biomarkers provide insights into the different pathways involved in HFPEF. **Panel A** represents a patient centered summary of the different type of biomarker pathways assessed in our study as well as in other studies. **Panel B** represents a partial regression network of biomarkers emerging in the PLS-DA. Notable connections include the connections between the IGFBP-1 and GDF-15, IGFBP-7 and angiopoetin-2, GCSF and PDGF; in addition, GDF-15 was

more strongly linked to ICAM-1 and FGF-21. Abbreviations as per previous figures and supplementary table 1.

Table 1. Patient characteristics of the INSPIRE Registry

Variables	**HFpEF (n=96)**	**Controls (n=96)**	***p* value**
Age, years	74.4 [68.3; 83.7]	72.9 [66.1; 78.8]	0.14
Male sex	39 (40.6)	42 (43.8)	0.66
White race	91 (94.8)	94 (97.9)	0.25
Body mass index BMI, kg/m2	30.5 [25.6; 37.9]	31.2 [27.4; 34.8]	0.75
Hemodynamics			
Heart rate (bpm)	71.0 [63.5; 86.0]	69.0 [61.0; 79.0]	0.09
Systolic Blood Pressure, mmHg	140.0 [121.5; 154.0]	142.0 [124.0; 154.0]	0.84
Diastolic Blood Pressure, mmHg	74.0 [62.5; 83.5]	75.0 [64.0; 83.0]	0.55
Comorbidity history			
Systemic Hypertension, n (%)	93 (96.9)	83 (86.5)	<0.01
Diabetes mellitus, n (%)	32 (33.3)	30 (31.3)	0.76
Dyslipidemia, n (%)	81 (84.4)	61 (63.5)	<0.01

Coronary artery disease, n (%)	71 (74.0)	66 (68.8)	0.42
Cerebrovascular disease, n (%)	8 (8.3)	4 (4.2)	0.23
Atrial fibrillation history, , n (%)	67 (69.8)	19 (19.8)	<0.001
Non-skin cancer history, , n (%)	41 (42.7)	26 (27.1)	0.02
Chronic obstructive pulmonary disease, n (%)	19 (19.8)	2 (2.1)	<0.001
History of depression, n (%)	31 (32.3)	17 (17.7)	0.02
Intermountain Risk Score	n=91 13.0 [11.0; 15.0]	n=63 11.0 [9.0; 13.0]	<0.01
Medications			
Beta Blocker	63 (65.6)	40 (41.7)	<0.001
Calcium-channel blocker	34 (35.4)	12 (12.5)	<0.001
Diuretic	84 (87.5)	28 (29.2)	<0.001
ACE inhibitor	35 (36.5)	33 (34.4)	0.76
ARB	22 (22.9)	13 (13.5)	0.09
Aldosterone inhibitor	16 (16.7)	0	<0.001
Antiplatelet therapy	67 (69.8)	62 (64.6)	0.44
Warfarin or NOAC	45 (46.9)	7 (7.3)	<0.001
Statin	58 (60.4)	45 (46.9)	0.06
Echocardiographic data			

LV ejection fraction (%)	60.0 [55.5; 65.0]	63.0 [60.0; 65.0] n=71	0.03
Right ventricular systolic pressure (mmHg)	49.3 [40.9; 60.5] n=55	23.9 [19.4; 32.1] n=16	<0.001
Laboratory data			
Hemoglobin (g/dL)	12.7 [11.3; 14.0]	13.9 [13.1; 15.0]	<0.001
eGFR MDRD (mL/min/1.73m2)	64.4 [46.9; 74.2]	70.8 [48.0; 79.7] n=63	0.28

Data is presented as median and [interquartile range] or number (percentage). Continuous data is compared using Mann-Whitney test while categorical data is compared using Chi-square test. ACE inhibitor: angiotensin-converting enzyme inhibitor; ARB: angiotensin II receptor blocker; eGFR: estimated glomerular filtration rate using the MDRD formula; NOAC: novel oral anticoagulant.

Table 2. Association with HFpEF and all-cause mortality in the CATHGEN cohort.

A. Odds ratios for association with the presence of HFpEF

IGF axis	Univariate model		Multivariable model	
	OR (95% CI)	p	OR (95% CI)	p
IGF-2R	1.19 (0.88-1.63)	0.26	1.03 (0.72-1.48)	0.86
IGFBP-1	1.48 (1.09-2.04)	0.014	1.43 (0.92-2.25)	0.12
IGFBP-2	1.66 (1.21-2.33)	0.002	1.29 (0.83-2.05)	0.26
IGFBP-3	0.5 (0.34-0.71)	<0.001	0.64 (0.42-0.97)	0.038
IGFBP-6	1.68 (1.21-2.43)	0.004	0.62 (0.34-1.09)	0.10
IGFBP-7	2.17 (1.51-3.22)	5.95×10^{-5}	1.49 (0.99-2.32)	0.060
IGFBPL-1	2.66 (1.83-4.06)	1.28×10^{-6}	1.75 (1.04-3.03)	0.039

B. Hazard ratios for time to all-cause mortality, truncated at 5 years

IGF axis	Univariate model		Multivariable model	
	HR (95% CI)	p	HR (95% CI)	p
IGF-2R	1.12 (0.76-1.63)	0.57	0.92 (0.56-1.5)	0.73
IGFBP-1	2.17 (1.45-3.23)	1.5×10^{-4}	2.15 (1.27-3.66)	0.0045
IGFBP-2	2.3 (1.53-3.46)	6.1×10^{-5}	2.26 (1.34-3.79)	0.0021

IGFBP-3	0.75 (0.55-1.02)	0.068	0.63 (0.46-0.87)	0.0042
IGFBP-6	1.25 (0.88-1.79)	0.22	0.78 (0.49-1.24)	0.30
IGFBP-7	1.43 (1.04-1.95)	0.026	1.19 (0.83-1.69)	0.34
IGFBPL-1	1.60 (1.12-2.27)	0.0090	1.45 (0.94-2.23)	0.096

Abbreviations as per text. Multivariable model was adjusted for age, sex, race, BMI, systolic BP, creatinine, and diabetes mellitus status