SUPPLEMENTARY MATERIAL

Proteomic Evaluation of the Comorbidity-Inflammation Paradigm in Heart Failure with Preserved Ejection Fraction:

Results from the PROMIS-HFpEF Study

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SUPPLEMENTAL METHODS

Biomarker measurement and internal validation

The Olink Proseek multiplex immunoassay is based on a proximity extension assay, using target-specific antibody pairs that are linked to DNA strands, creating a real-time polymerase chain reaction amplicon upon binding to the target analyte in a proximity-dependent manner, enabled by the action of a DNA polymerase. The intra-assay coefficient of variation (CV) ranges between 5% and 13% (mean 8%); the inter-assay CV ranges between 9% and 39% (mean 15%). Batch-effects were excluded and random partitioning of samples was confirmed. Ten biomarkers were measured on 2 panels (CCL3, CXCL1, OPG, IL-6, uPA, MCP1, SCF, IL-18, FGF-21, FGF-23). The correlation between panels for these paired-biomarkers ranged from 0.87 to 0.97 in both cohorts. For further analysis, we randomly selected 1 panel for each of these paired-biomarkers. The correlation between NTproBNP using Olink-technology and using a commercially available ELISA immunoassay (Elecsys® NT-proBNP assay, Roche Diagnostics GmbH, Mannheim) was 0.89 ($P<0.001$) in both cohorts. When biomarker levels fell below limit of detection (LOD) in >=50% of the study population, they were excluded from further analysis (n=18: IL-20RA, IL-2RB, IL-1 alpha, IL-2, TSLP, FGF-5, IL-22RA1, IL-24, IL-13, ARTN, TNF, IL-20, IL-33, IFN-gamma, IL4, LIF, NRTN, IL-5).

Linear regression models

The main assumptions of linear regression where checked (i.e. linearity, homogeneity of variance, normal distribution of residuals) by evaluating Q-Q plots and scatterplots of fitted values versus residuals. Regression models with the endpoint of LA volume and LA volume index showed non-normal distributed Q-Q plots. Log-transformation was attempted, but did not change overall results in terms of R^2 or p-value. Therefore and for easier interpretability, non-transformed results are reported. Outliers were evaluated using standardized and studentized residuals and influential cases were evaluated by Cook's distance, Hat-values, DFbeta and DFFit; none of models suffered from relevant outliers or influential cases.

Confounders

We evaluated several variables based on clinical insight and based on baseline differences by comorbidity burden (i.e. age, gender, GFR, atrial fibrillation, NYHA-class, inflammatory disease and/or malignancy, active malignancy, BMI, use of a statin and use of a loop diuretic) and found that age, gender, and GFR were potential confounders for the association between comorbidity burden and cardiac structure & function and/or between comorbidity burden and inflammation. Additionally, atrial fibrillation was a confounder for regression analyses including e' velocity, E/e' ratio and left atrial parameters as an endpoint. Active malignancy was a potential confounder for regression analyses including PC6 and the yellow cluster, inflammatory disease and previous malignancy were not, but we corrected for them in the regressions for inflammation anyway. Study site was entered as a dummy variable in all regression and mediation analyses because of the many differences in baseline characteristics by study site (e.g. BMI, PC6, yellow-ME, LV-dimensions, LAVI, LA-strain, GLS, e' velocity, RV-dimensions, RV-function)

Principal component analysis (PCA) of inflammation markers

After mean centering and scaling, rotated PCA (oblimin, package 'psych') was performed in order to extract principal components (PCs) and as a means of data reduction. Rotation maximizes the loading of biomarkers on a single PC while minimizing the loading on all other PCs. The oblique method was chosen because the inflammatory biomarker PCs are expected to be correlated (and not fully orthogonal) and because rotation improves factor interpretability. Six rotated principal components (PCs) were extracted, as suggested by the scree plot in unrotated PCA. These rotated components respectively explained 19%, 10%, 8%, 7%, 5% and 8% of biomarker variation. Rotated PC1 and PC6 had a correlation of 0.41, other PCs correlated <0.30.

Functional classification and pathway overrepresentation analysis in Metascape:

Biomarkers were entered into the online Metascape application according to their corresponding Entez Gene ID (see supplemental table I) and analyzed as 'homo sapiens'. For both membership analysis and pathway overrepresentation analysis, three databases were used: i) Gene Ontology (GO), the primary data source for enrichment analysis, using the ontology branch "Biological Process" (BP), http://geneontology.org; ii) Kyoto Encyclopedia of Genes and Genomes (KEGG), similar and complementary to GO, also contains some compound data, http://www.genome.jp/kegg; and iii) Reactome, a curated and peer reviewed pathway database used for enrichment analysis, http://www.reactome.org (accessed on March 18, 2019). Memebership analysis was preformed applying keyword searches followed by manual selection of specific pathways to the total available list of Olink-biomarkers in each of these databases (supplemental table II). Biomakers that were annotated to any of these selected pathways in three out of three databases were finally selected. Pathway overrepresentation analysis was performed applying the standard accumulative hypergeometric statistical test to identify pathways where input genes show significant presence, automatically clustering resultant terms to reduce redundancy. The total included list of Olink-biomarkers was used as a background-list instead of using the whole genome, since our study was conducted using a functionally biased protein-collection. For small clusters(<20 biomarkers), an enriched protein-protein-interaction network was additionally constructed using the STRING database (a database of known and predicted interactions based on the literature, experimental data and computational prediction methods) and the following parameters: confidence cut-off = 0.80, maximum added interactors = 15.

Weighted coexpression network analysis

Using the WGCNA package in R, weighted correlations were calculated for all pairs of biomarkers, creating a signed similarity matrix. The similarity matrix was then raised to the power β of 6 to approximate a scale-free topology of the topological overlap connectivity (k) of the entire network. This is performed to emphasize strong correlations and reduce the emphasis of weak correlations on an exponential scale. The resulting adjacency matrix is then transformed into a topological overlap matrix (TOM). Using TOM as the distance measure, biomarkers were hierarchically clustered, and clusters were determined using a dynamic tree-cutting algorithm (cutreeHybrid, using the following parameters except deepSplit = 4, cutHeight = 0.25, minClusterSize = 10, reassignThreshold = 0, other settings are default). The resulting clusters or clusters of coexpressed biomarkers were used to calculate the MEs, i.e. the first principal component of the cluster. Cluster preservation analysis was performed by first constructing WCNA networks and clusters in the Northwestern-HFpEF and Northwestern-control dataset individually and independently. Cluster hubs were defined by calculating in-cluster protein connectivity, which represent the sum of the weighed correlations with all biomarkers in the cluster. Cluster membership (kME) - which are the Pearson correlations between each biomarker and each ME - was also calculated and used in network plots. Network comparative analysis were done using a hypergeometric distribution, testing if the number of overlapping biomarkers between one dataset and a given cluster was significantly large with any cluster in the other dataset. Accordingly, corresponding clusters were assigned the same color.

SUPPLEMENTARY TABLES

Supplementary Table I: Full listing of the Olink proteins included in the study (CVD2, CVD3, and Inflammation panels)

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
ACE2	ACE2	59272	Angiotensin-converting enzyme 2 (ACE2)		—	turquoise	—
ADA	ADA	100	Adenosine Deaminase (ADA)		blue	blue	—
ADAMTS13	ADAMTS13	11093	A disintegrin and metalloproteinase with thrombospondin motifs 13 (ADAM-TS13)		brown	blue	blue
ADM	ADM	133	ADM (ADM)		brown	turquoise	turquoise3
AGRP	AGRP	181	Agouti-related protein (AGRP)		brown	turquoise	—
ALCAM	ALCAM	214	CD166 antigen (ALCAM)		yellow	turquoise	turquoise2
AMBP	AMBP	259	Protein AMBP (AMBP)		brown	turquoise	turquoise3
ANG1	ANGPT1	284	Angiopoietin-1 (ANG-1)		blue	purple	purple
APN	ANPEP	290	Aminopeptidase N (AP-N)		—	turquoise	—
AXIN1	AXIN1	8312	Axin-1 (AXIN1)		blue	blue	blue
AXL	AXL	558	Tyrosine-protein kinase receptor UFO (AXL)		turquoise	turquoise	turquoise2
AZU1	AZU1	566	Azurocidin (AZU1)		turquoise	orange	orange
BetaNGF	NGF	4803	Beta-nerve growth factor (Beta-NGF)		—	turquoise	—
BLMH	BLMH	642	Bleomycin hydrolase (BLM hydrolase)		blue	blue	blue
BMP6	BMP6	654	Bone morphogenetic protein 6 (BMP—6)		brown	turquoise	turquoise2

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
BNP	NPPB	4879	Natriuretic peptides B (BNP)		—	turquoise	—
BOC	BOC	91653	Brother of CDO (Protein BOC)		brown	turquoise	purple
CA5A	CA5A	763	Carbonic anhydrase 5A, mitochondrial (CA5A)		—	turquoise	—
CASP3	CASP3	836	Caspase-3 (CASP-3)		blue	blue	blue
CASP8	CASP8	841	Caspase 8 (CASP-8)	X	blue	blue	orange
CCL11	CCL11	6356	Eotaxin-1 (CCL11)	X	—	—	—
CCL15	CCL15	6359	C-C motif chemokine 15 (CCL15)		turquoise	turquoise	—
CCL16	CCL16	6360	C-C motif chemokine 16 (CCL16)		—	—	—
CCL17	CCL17	6361	C-C motif chemokine 17 (CCL17)		blue	purple	purple
CCL19	CCL19	6363	C-C motif chemokine 19 (CCL19)	X	—	turquoise	—
CCL20	CCL20	6364	C-C motif chemokine 20 (CCL20)	X	—	turquoise	blue
CCL23	CCL23	6368	C-C motif chemokine 23 (CCL23)		turquoise	turquoise	—
CCL24	CCL24	6369	C-C motif chemokine 24 (CCL24)		—	—	—
CCL25	CCL25	6370	C-C motif chemokine 25 (CCL25)		—	turquoise	blue
CCL28	CCL28	56477	C-C motif chemokine 28 (CCL28)		—	—	green
CCL3	CCL3	6348	C-C motif chemokine 3 (CCL3)	X	brown	turquoise	—
CCL4	CCL4	6351	C-C motif chemokine 4 (CCL4)	X	—	purple	—
CD163	CD163	9332	Scavenger receptor cysteine-rich type 1 protein M130 (CD163)		yellow	turquoise	turquoise2

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
CD244	CD244	51744	Natural killer cell receptor 2B4 (CD244)		blue	turquoise	blue
CD4	CD4	920	T-cell surface glycoprotein CD4 (CD4)	X	brown	turquoise	turquoise3
CD40	CD40	958	CD40L receptor (CD40)	X	turquoise	turquoise	blue
CD40L	CD40LG	959	CD40 ligand (CD40-L)	X	blue	purple	purple
CD5	CD5	921	T-cell surface glycoprotein CD5 (CD5)		turquoise	turquoise	turquoise
CD6	CD6	923	T cell surface glycoprotein CD6 isoform (CD6)		—	turquoise	turquoise
CD84	CD84	8832	SLAM family member 5 (CD84)		brown	purple	purple
CD8A	CD8A	925	T-cell surface glycoprotein CD8-alpha (CD8A)	X	—	n.a.	n.a.
CD93	CD93	22918	Complement component C1q receptor (CD93)		turquoise	turquoise	turquoise
CDCP1	CDCP1	64866	CUB domain-containing protein 1 (CDCP1)		turquoise	turquoise	—
CDH5	CDH5	1003	Cadherin-5 (CDH5)		yellow	turquoise	turquoise2
CEACAM8	CEACAM8	1088	Carcinoembryonic antigenrelated cell adhesion molecule 8 (CEACAM8)		turquoise	orange	orange
CHI3L1	CHI3L1	1116	Chitinase-3-like protein 1 (CHI3L1)		turquoise	turquoise	—
CHIT1	CHIT1	1118	Chitotriosidase-1 (CHIT1)		—	—	—
CNTN1	CNTN1	1272	Contactin-1 (CNTN1)		yellow	—	turquoise2

Protein abbreviation	**Gene name**	**Entez ID**	**Protein full name**	**Selected as inflammation-related**	**Assigned cluster**		
					PROMIS HFpEF	**Northwestern HFpEF**	**Northwestern Controls**
COL1A1	COL1A1	1277	Collagen I alpha-1 chain (COL1A1)		—	turquoise	green
CPA1	CPA1	1357	Carboxypeptidase A1 (CPA1)		—	—	—
CPB1	CPB1	1360	Carboxypeptidase B (CPB1)		—	—	—
CSF1	CSF1	1435	Macrophage colony-stimulating factor 1 (CSF-1)	X	turquoise	turquoise	turquoise
CST5	CST5	1473	Cystatin D (CST5)		—	turquoise	—
CSTB	CSTB	1476	Cystatin-B (CSTB)		turquoise	turquoise	turquoise3
CTRC	CTRC	11330	Chymotrypsin C (CTRC)		—	—	—
CTSD	CTSD	1509	Cathepsin D (CTSD)		—	turquoise	turquoise
CTSL1	CTSL	1514	Cathepsin L1 (CTSL1)		brown	turquoise	—
CTSZ	CTSZ	1522	Cathepsin Z (CTSZ)		turquoise	turquoise	turquoise
CX3CL1	CX3CL1	6376	Fractalkine (CX3CL1)		turquoise	turquoise	turquoise
CXCL1	CXCL1	2919	C-X-C motif chemokine 1 (CXCL1)	X	blue	—	turquoise2
CXCL10	CXCL10	3627	C-X-C motif chemokine 10 (CXCL10)	X	—	turquoise	green
CXCL11	CXCL11	6373	C-X-C motif chemokine 11 (CXCL11)		blue	—	—
CXCL16	CXCL16	58191	C-X-C motif chemokine 16 (CXCL16)		turquoise	turquoise	turquoise2
CXCL5	CXCL5	6374	C-X-C motif chemokine 5 (CXCL5)		blue	purple	turquoise2
CXCL6	CXCL6	6372	C-X-C motif chemokine 6 (CXCL6)		blue	—	purple

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
CXCL9	CXCL9	4283	C-X-C motif chemokine 9 (CXCL9)		—	turquoise	green
DCN	DCN	1634	Decorin (DCN)		brown	turquoise	turquoise3
DECR1	DECR1	1666	2,4-dienoyl-CoA reductase, mitochondrial (DECR1)		blue	blue	blue
Dkk1	DKK1	22943	Dickkopf-related protein 1 (Dkk-1)		blue	purple	purple
DLK1	DLK1	8788	Protein delta homolog 1 (DLK-1)		turquoise	turquoise	turquoise
DNER	DNER	92737	Delta and Notch-like epidermal growth factor- related receptor (DNER)		—	—	—
EGFR	EGFR	1956	Epidermal growth factor receptor (EGFR)		turquoise	—	—
EIF4EBP1	EIF4EBP1	1978	Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)		blue	blue	blue
ENRAGE	S100A12	6283	Protein S100-A12 (EN-RAGE)		—	orange	orange
EpCAM	EPCAM	4072	Epithelial cell adhesion molecule (Ep-CAM)		—	—	—
EPHB4	EPHB4	2050	Ephrin type-B receptor 4 (EPHB4)		turquoise	turquoise	turquoise
FABP2	FABP2	2169	Fatty acid-binding protein, intestinal (FABP2)		—	—	turquoise3
FABP4	FABP4	2167	Fatty acid-binding protein, adipocyte (FABP4)		turquoise	turquoise	—
FABP6	FABP6	2172	Gastrotropin (GT)		—	turquoise	orange
FAS	FAS	355	Tumor necrosis factor receptor		turquoise	turquoise	turquoise

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
			superfamily member 6 (FAS)				
FGF19	FGF19	9965	Fibroblast growth factor 19 (FGF-19)		—	—	—
FGF21	FGF21	26291	Fibroblast growth factor 21 (FGF-21)		—	turquoise	—
FGF23	FGF23	8074	Fibroblast growth factor 23 (FGF-23)		turquoise	turquoise	blue
Flt3L	FLT3LG	2323	Fms-related tyrosine kinase 3 ligand (Flt3L)		—	—	green
FS	FST	10468	Follistatin (FS)		brown	—	green
Gal3	LGALS3	3958	Galectin-3 (Gal-3)		turquoise	turquoise	turquoise3
Gal4	LGALS4	3960	Galectin-4 (Gal-4)		—	turquoise	turquoise3
Gal9	LGALS9	3965	Galectin-9 (Gal-9)		turquoise	turquoise	turquoise3
GDF15	GDF15	9518	Growth/differentiation factor 15 (GDF-15)		turquoise	turquoise	turquoise3
GDF2	GDF2	2658	Growth/differentiation factor 2 (GDF-2)		—	—	purple
GDNF	GDNF	2668	Glial cell line-derived neurotrophic factor (GDNF)		—	—	green
GH	GH1	2688	Growth hormone (GH)		—	—	—
GIF	GIF	2694	Gastric intrinsic factor (GIF)		—	—	—
GLO1	GLO1	2739	Lactoylglutathione lyase (GLO1)		blue	—	orange
GP6	GP6	51206	platelet glycoprotein VI (GP6)		blue	n.a.	n.a.
GRN	GRN	2896	Granulins (GRN)		yellow	turquoise	turquoise2
HAOX1	HAO1	54363	Hydroxyacid oxidase 1 (HAOX1)		—	turquoise	—
HBEGF	HBEGF	1839	Proheparin-binding EGF-like growth factor (HB-EGF)		blue	purple	purple

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
HGF	HGF	3082	Hepatocyte growth factor (HGF)	X	yellow	—	green
HO1	HMOX1	3162	Heme oxygenase 1 (HO-1)		brown	turquoise	—
hOSCAR	OSCAR	126014	Osteoclast-associated immunoglobulin-like receptor (hOSCAR)		brown	turquoise	turquoise3
HSP27	HSPB1	3315	Heat shock 27 kDa protein (HSP 27)		blue	blue	blue
ICAM2	ICAM2	3384	Intercellular adhesion molecule 2 (ICAM-2)	X	—	turquoise	turquoise
IDUA	IDUA	3425	Alpha-L-iduronidase (IDUA)		—	—	—
IGFBP1	IGFBP1	3484	Insulin-like growth factor-binding protein 1 (IGFBP-1)		—	turquoise	—
IGFBP2	IGFBP2	3485	Insulin-like growth factor-binding protein 2 (IGFBP-2)		—	turquoise	—
IGFBP7	IGFBP7	3490	Insulin-like growth factor-binding protein 7 (IGFBP-7)		yellow	turquoise	turquoise
IgGFcR2B	FCGR2B	2213	Low affinity immunoglobulin gamma Fc region receptor II-b (IgG Fc receptor II-b)		—	—	green
IL10	IL10	3586	Interleukin-10 (IL-10)	X	—	turquoise	—
IL10RA	IL10RA	3587	Interleukin-10 receptor subunit alpha (IL-10RA)		—	—	—
IL10RB	IL10RB	3588	Interleukin-10 receptor subunit beta (IL-10RB)	X	turquoise	turquoise	turquoise
IL12B	IL12B	3593	Interleukin-12 subunit beta (IL-12B)	X	turquoise	turquoise	green

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
IL15RA	IL15RA	3601	Interleukin-15 receptor subunit alpha (IL-15RA)		—	turquoise	turquoise
IL16	IL16	3603	Pro-interleukin-16 (IL16)		brown	orange	orange
IL17A	IL17A	3605	Interleukin-17A (IL-17A)	X	—	—	—
IL17C	IL17C	27189	Interleukin-17C (IL-17C)	X	—	turquoise	—
IL17D	IL17D	53342	Interleukin-17D (IL-17D)		—	—	green
IL17RA	IL17RA	23765	Interleukin-17 receptor A (IL-17RA)	X	—	turquoise	—
IL18	IL18	3606	Interleukin-18 (IL-18)	X	brown	turquoise	—
IL18BP	IL18BP	10068	Interleukin-18-binding protein (IL-18BP)		turquoise	turquoise	turquoise
IL18R1	IL18R1	8809	Interleukin-18 receptor 1 (IL-18R1)	X	—	turquoise	—
IL1RA	IL1RN	3557	Interleukin-1 receptor antagonist protein (IL-1ra)		turquoise	orange	turquoise3
IL1RL2	IL1RL2	8808	Interleukin-1 receptor-like 2 (IL1RL2)		—	—	—
IL1RT1	IL1R1	3554	Interleukin-1 receptor type 1 (IL-1RT1)	X	yellow	turquoise	turquoise2
IL1RT2	IL1R2	7850	Interleukin-1 receptor type 2 (IL-1RT2)	X	—	—	—
IL27	IL27	246778	Interleukin-27 (IL-27)		brown	turquoise	—
IL2RA	IL2RA	3559	Interleukin-2 receptor subunit alpha (IL2-RA)	X	turquoise	turquoise	turquoise
IL4RA	IL4R	3566	Interleukin-4 receptor subunit alpha (IL-4RA)	X	turquoise	turquoise	turquoise3
IL6	IL6	3569	Interleukin-6 (IL-6)	X	—	—	—

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
IL6RA	IL6R	3570	Interleukin-6 receptor subunit alpha (IL6-RA)	X	—	—	—
IL7	IL7	3574	Interleukin-7 (IL-7)		blue	purple	purple
IL8	CXCL8	3576	Interleukin-8 (IL-8)	X	—	turquoise	purple
ITGB1BP2	ITGB1BP2	26548	Melusin (ITGB1BP2)		blue	blue	blue
ITGB2	ITGB2	3689	Integrin beta-2 (ITGB2)	X	—	—	turquoise2
JAMA	F11R	50848	Junctional adhesion molecule A (JAM-A)		blue	blue	blue
KIM1	HAVCR1	26762	Kidney injury molecule 1 (KIM-1)		—	turquoise	turquoise3
KLK6	KLK6	5653	Kallikrein-6 (KLK6)		turquoise	turquoise	turquoise
LAPTGFb1	TGFB1	7040	Latency-associated peptide transforming growth factor beta 1 (LAP TGF-beta-1)	X	blue	purple	purple
LDLreceptor	LDLR	3949	Low-density lipoprotein receptor (LDL receptor)		—	—	—
LEP	LEP	3952	Leptin (LEP)		—	—	—
LIFR	LIFR	3977	Leukemia inhibitory factor receptor (LIF-R)		yellow	turquoise	turquoise2
LOX1	OLR1	4973	Lectin-like oxidized LDL receptor 1 (LOX-1)		blue	orange	orange
LPL	LPL	4023	Lipoprotein lipase (LPL)		brown	turquoise	turquoise
LTBR	LTBR	4055	Lymphotoxin-beta receptor (LTBR)	X	turquoise	turquoise	turquoise
MARCO	MARCO	8685	Macrophage receptor MARCO (MARCO)		brown	turquoise	—
MB	MB	4151	Myoglobin (MB)		turquoise	turquoise	—
MCP1	CCL2	6347	Monocyte chemotactic protein 1 (MCP-1)	X	—	purple	purple

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
MCP2	CCL8	6355	Monocyte chemotactic protein 2 (MCP-2)		—	purple	purple
MCP3	CCL7	6354	Monocyte chemotactic protein 3 (MCP-3)		—	orange	—
MCP4	CCL13	6357	Monocyte chemotactic protein 4 (MCP-4)		blue	purple	—
MEPE	MEPE	56955	Matrix extracellular phosphoglycoprotein (MEPE)		turquoise	turquoise	turquoise
MERTK	MERTK	10461	Tyrosine-protein kinase Mer (MERTK)		brown	turquoise	purple
MMP1	MMP1	4312	Matrix metalloproteinase-1 (MMP-1)		blue	—	purple
MMP10	MMP10	4319	Matrix metalloproteinase-10 (MMP-10)		—	turquoise	turquoise3
MMP12	MMP12	4321	Matrix metalloproteinase-12 (MMP-12)		brown	—	turquoise3
MMP2	MMP2	4313	Matrix metalloproteinase-2 (MMP-2)		yellow	turquoise	turquoise2
MMP3	MMP3	4314	Matrix metalloproteinase-3 (MMP-3)	X	—	turquoise	—
MMP7	MMP7	4316	Matrix metalloproteinase-7 (MMP-7)		brown	blue	blue
MMP9	MMP9	4318	Matrix metalloproteinase-9 (MMP-9)	X	blue	orange	orange
MPO	MPO	4353	Myeloperoxidase (MPO)		—	orange	orange
NEMO	IKBKG	8517	NF-kappa-B essential modulator (NEMO)	X	blue	blue	blue

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
Notch3	NOTCH3	4854	Neurogenic locus notch homolog protein 3 (Notch 3)		yellow	turquoise	turquoise2
NT3	NTF3	4908	Neurotrophin-3 (NT-3)		—	—	blue
NTproBNP	NPPB	4879	N-terminal prohormone brain natriuretic peptide (NT-pro BNP)		—	turquoise	—
OPG	TNFRSF11B	4982	Osteoprotegerin (OPG)		yellow	turquoise	blue
OPN	SPP1	6696	Osteopontin (OPN)		turquoise	turquoise	—
OSM	OSM	5008	Oncostatin-M (OSM)	X	blue	orange	orange
PAI	SERPINE1	5054	Plasminogen activator inhibitor 1 (PAI)		blue	purple	purple
PAPPA	PAPPA	5069	Pappalysin-1 (PAPPA)		—	—	green
PAR1	F2R	2149	Proteinase-activated receptor 1 (PAR-1)		brown	blue	blue
PARP1	PARP1	142	Poly [ADP-ribose] polymerase 1 (PARP-1)		—	blue	blue
PCSK9	PCSK9	255738	Proprotein convertase subtilisin/kexin type 9 (PCSK9)		—	turquoise	turquoise
PDGFA	PDGFA	5154	Platelet-derived growth factor subunit A (PDGF subunit A)		blue	purple	purple
PDGFB	PDGFB	5155	Platelet-derived growth factor subunit B (PDGF subunit B)		blue	purple	purple
PDL1	CD274	29126	Programmed cell death 1 ligand 1 (PD-L1)	X	turquoise	turquoise	turquoise
PDL2	PDCD1LG2	80380	Programmed cell death 1 ligand 2 (PD-L2)		brown	turquoise	purple

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
PECAM1	PECAM1	5175	Platelet endothelial cell adhesion molecule (PECAM-1)		blue	blue	blue
PGLYRP1	PGLYRP1	8993	Peptidoglycan recognition protein 1 (PGLYRP1)		turquoise	orange	orange
PI3	PI3	5266	Elafin (PI3)		turquoise	turquoise	—
PIgR	PIGR	5284	Polymeric immunoglobulin receptor (PIgR)		brown	blue	purple
PLC	HSPG2	3339	Perlecan (PLC)		turquoise	turquoise	turquoise
PLGF	PGF	5228	Placenta growth factor (PlGF)		brown	turquoise	turquoise3
PON3	PON3	5446	Paraoxonase (PON3)		—	—	—
PRELP	PRELP	5549	Prolargin (PRELP)		brown	turquoise	turquoise3
PRSS27	PRSS27	83886	Serine protease 27 (PRSS27)		brown	—	purple
PRSS8	PRSS8	5652	Prostasin (PRSS8)		brown	turquoise	turquoise3
PRTN3	PRTN3	5657	Myeloblastin (PRTN3)		turquoise	orange	orange
PSGL1	SELPLG	6404	P-selectin glycoprotein ligand 1 (PSGL-1)		—	—	—
PSPD	SFTPD	6441	Pulmonary surfactant-associated protein D (PSP-D)		—	—	—
PTX3	PTX3	5806	Pentraxin-related protein PTX3 (PTX3)		—	orange	—
RAGE	AGER	177	Receptor for advanced glycosylation end products (RAGE)		brown	turquoise	green
RARRES2	RARRES2	5919	Retinoic acid receptor responder protein 2 (RARRES2)		turquoise	—	—
REN	REN	5972	Renin (REN)		brown	—	—
RETN	RETN	56729	Resistin (RETN)		turquoise	orange	turquoise

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
SCF	KITLG	4254	Stem cell factor (SCF)		brown	—	—
SCGB3A2	SCGB3A2	117156	Secretoglobin family 3A member 2 (SCGB3A2)		—	—	green
SELE	SELE	6401	E-selectin (SELE)		—	—	turquoise
SELP	SELP	6403	P-selectin (SELP)		blue	blue	blue
SERPINA12	SERPINA12	145264	Serpin A12 (SERPINA12)		—	—	—
SHPS1	SIRPA	140885	Tyrosine-protein phosphatase non-receptor type substrate 1 (SHPS-1)		turquoise	turquoise	turquoise
SIRT2	SIRT2	22933	SIR2-like protein 2 (SIRT2)		blue	blue	blue
SLAMF1	SLAMF1	6504	Signaling lymphocytic activation molecule (SLAMF1)		—	turquoise	—
SLAMF7	SLAMF7	57823	SLAM family member 7 (SLAMF7)		—	turquoise	green
SOD2	SOD2	6648	Superoxide dismutase [Mn], mitochondrial (SOD2)		brown	—	blue
SORT1	SORT1	6272	Sortilin (SORT1)		brown	purple	purple
SPON1	SPON1	10418	Spondin-1 (SPON1)		yellow	—	green
SPON2	SPON2	10417	Spondin-2 (SPON2)		brown	blue	turquoise3
SRC	SRC	6714	Proto-oncogene tyrosine-protein kinase Src (SRC)	X	blue	blue	blue
ST1A1	SULT1A1	6817	Sulfotransferase 1A1 (ST1A1)		blue	blue	blue
ST2	IL1RL1	9173	ST2 protein (ST2)		—	turquoise	—
STAMPB	STAMBP	10617	STAM-binding protein (STAMBP)		blue	blue	blue
STK4	STK4	6789	Serine/threonine-protein kinase 4 (STK4)		blue	blue	blue

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
TF	F3	2152	Tissue factor (TF)		brown	turquoise	purple
TFF3	TFF3	7033	Trefoil factor 3 (TFF3)		turquoise	turquoise	turquoise3
TFPI	TFPI	7035	Tissue factor pathway inhibitor (TFPI)		—	—	green
TGFa	TGFA	7039	Transforming growth factor alpha (TGF-alpha)		turquoise	orange	orange
TGM2	TGM2	7052	Protein-glutamine gamma-glutamyltransferase 2 (TGM2)		blue	blue	blue
THBS2	THBS2	7058	Thrombospondin-2 (THBS2)		—	—	—
THPO	THPO	7066	Thrombopoietin (THPO)		blue	purple	purple
TIE2	TEK	7010	Angiopoietin-1 receptor (TIE2)		brown	turquoise	purple
TIMP4	TIMP4	7079	Metalloproteinase inhibitor 4 (TIMP4)		—	turquoise	—
TLT2	TREML2	79865	Trem-like transcript 2 protein (TLT-2)		turquoise	turquoise	turquoise
TM	THBD	7056	Thrombomodulin (TM)		brown	turquoise	purple
TNFB	LTA	4049	TNF-beta (TNFB)	X	—	turquoise	blue
TNFR1	TNFRSF1A	7132	Tumor necrosis factor receptor 1 (TNF-R1)	X	turquoise	turquoise	turquoise
TNFR2	TNFRSF1B	7133	Tumor necrosis factor receptor 2 (TNF-R2)	X	turquoise	turquoise	turquoise
TNFRSF10A	TNFRSF10A	8797	Tumor necrosis factor receptor superfamily member 10A (TNFRSF10A)		brown	turquoise	turquoise3
TNFRSF10C	TNFRSF10C	8794	Tumor necrosis factor receptor		turquoise	orange	turquoise

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
			superfamily member 10C (TNFRSF10C)				
TNFRSF11A	TNFRSF11A	8792	Tumor necrosis factor receptor superfamily member 11A (TNFRSF11A)	X	brown	turquoise	turquoise3
TNFRSF13B	TNFRSF13B	23495	Tumor necrosis factor receptor superfamily member 13B (TNFRSF13B)		brown	turquoise	—
TNFRSF14	TNFRSF14	8764	Tumor necrosis factor receptor superfamily member 14 (TNFRSF14)	X	turquoise	turquoise	turquoise
TNFRSF9	TNFRSF9	3604	Tumor necrosis factor receptor superfamily member 9 (TNFRSF9)		turquoise	turquoise	turquoise
TNFSF13B	TNFSF13B	10673	Tumor necrosis factor ligand superfamily member 13B (TNFSF13B)	X	—	turquoise	turquoise
TNFSF14	TNFSF14	8740	Tumor necrosis factor ligand superfamily member 14 (TNFSF14)		blue	orange	orange
tPA	PLAT	5327	Tissue-type plasminogen activator (t-PA)		blue	—	—
TR	TFRC	7037	Transferrin receptor protein 1 (TR)		—	turquoise	turquoise2
TRAIL	TNFSF10	8743	TNF-related apoptosis-inducing ligand (TRAIL)		—	—	blue
TRAILR2	TNFRSF10B	8795	TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2)		brown	turquoise	turquoise3

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
TRANCE	TNFSF11	8600	TNF-related activation-induced cytokine (TRANCE)	X	—	—	—
TRAP	ACP5	54	Tartrate-resistant acid phosphatase type 5 (TR-AP)		—	—	turquoise
TWEAK	TNFSF12	8742	Tumor necrosis factor (Ligand) superfamily, member 12 (TWEAK)		—	—	green
uPA	PLAU	5328	Urokinase-type plasminogen activator (uPA)		—	turquoise	blue
UPAR	PLAUR	5329	Urokinase plasminogen activator surface receptor (U-PAR)		turquoise	turquoise	turquoise
VEGFA	VEGFA	7422	Vascular endothelial growth factor A (VEGF-A)		turquoise	turquoise	purple
VEGFD	FIGF	2277	Vascular endothelial growth factor D (VEGF-D)		—	turquoise	green
VSIG2	VSIG2	23584	V-set and immunoglobulin domain-containing protein 2 (VSIG2)		—	turquoise	turquoise3
vWF	VWF	7450	von Willebrand factor (vWF)		blue	turquoise	—
XCL1	XCL1	6375	Lymphotactin (XCL1)		brown	turquoise	—
ARTN	ARTN	9048	Artemin (ARTN)				
FGF5	FGF5	2250	Fibroblast growth factor 5 (FGF-5)				
IFNg	IFNG	3458	Interferon gamma (IFN-gamma)	X			
IL13	IL13	3596	Interleukin-13 (IL-13)	X			
IL1a	IL1A	3552	Interleukin-1 alpha (IL-1 alpha)	X			

Protein abbreviation	Gene name	Entez ID	Protein full name	Selected as inflammation-related	Assigned cluster		
					PROMIS HFpEF	Northwestern HFpEF	Northwestern Controls
IL2	IL2	3558	Interleukin-2 (IL-2)	X			
IL20	IL20	50604	Interleukin-20 (IL-20)	X			
IL20RA	IL20RA	53832	Interleukin-20 receptor subunit alpha (IL-20RA)				
IL22RA1	IL22RA1	58985	Interleukin-22 receptor subunit alpha-1 (IL-22 RA1)				
IL24	IL24	11009	Interleukin-24 (IL-24)				
IL2RB	IL2RB	3560	Interleukin-2 receptor subunit beta (IL-2RB)				
IL33	IL33	90865	Interleukin-33 (IL-33)				
IL4	IL4	3565	Interleukin-4 (IL-4)	X			
IL5	IL5	3567	Interleukin-5 (IL-5)	X			
LIF	LIF	3976	Leukemia inhibitory factor (LIF)				
NRTN	NRTN	4902	Neurturin (NRTN)				
TNF	TNF	7124	Tumor necrosis factor (TNF)	X			
TSLP	TSLP	85480	Thymic stromal lymphopoietin (TSLP)	X			

Supplementary Table II: Search strategy and selected inflammatory pathways from GO, KEGG, and Reactome databases

Search term	Pathway description	Manually included pathways	Manually excluded pathways
GO > inflamm	GO Inflammation	Positive regulation, activation, production, response, regulation (neutral) of inflammation	Connective tissue
GO > immune	GO Immune response	>100 terms, all included	none
Reactome > immune	Reactome Immune system	Adaptive immune system, innate immune system, immune system	cytokine signaling, immune disease, RUNX3 regulates immune response and cell migration, SUMOxylation of immune response proteins
Reactome > immune	Reactome Cytokine signaling	Cytokine signaling (1280215)	all others
Reactome > inflamm	Not included		Resulted in only 1 specific pathway: CLe7a inflammasome
Reactome > leuko	No output		
reactome > inflammation OR inflammatory	No output		
KEGG > immune	KEGG Immune system	Th1, Th2 and TH17 differentiation, IL17 signaling, Toll-signaling, natural killer cytotoxicity, cell-adhesion molecules, NOD-signaling, chemokine signaling, leukocyte-endothelial migration	All others
KEGG > inflammatory	KEGG Cytokine signaling	Cytokine-cytokine receptor interaction (04060)	

KEGG > leuko	Not included		Resulted in only 1 specific pathway: leukocyte transendothelial migration
KEGG > inflamm	Not included		Inflammatory bowel disease, inflammatory mediation of TRP channels
KEGG > immun	Not included		Immunodeficiency, intestinal immune network
COMBINATIONS			
GO score	GO Immune response or Inflammation		
KEGG score	KEGG Immune system or Cytokine signaling		
Reactome score	Reactome Immune system or Cytokine signaling		
Total Immune / Inflammation score	1 point for each database (Immune or Inflammation/Cytokine)		
Double annotation	2 or more points on the total score		
Triple annotation	3 points on the total score		

GO = Gene Ontology, the primary data source for enrichment analysis, using the ontology branch "Biological Process" (BP), http://geneontology.org; KEGG = Kyoto Encyclopedia of Genes and Genomes, similar and complementary to GO, also contains some compound data, http://www.genome.jp/kegg; Reactome, a curated and peer reviewed pathway database used for enrichment analysis, http://www.reactome.org

Supplemental Table III. Top 10 Inflammatory Proteins in the Major Principal Components in PROMIS-HFpEF

PC1	Corr. coeff.	PC2	Corr. coeff.	PC3	Corr. coeff.	PC4	Corr. coeff.	PC5	Corr. coeff.	PC6	Corr. coeff.
TNFR1*†	0.91	CD40L	0.94	MMP9	0.92	TNFB	0.66	IL6	0.55	IL1RT1*†	0.75
LTBR*†	0.87	NEMO	0.93	OSM	0.87	CCL19	0.63	IL4RA	0.36	ICAM2†	0.61
TNFRSF14*†	0.83	SRC	0.84	MCP1	0.50	IL12B	0.60	CCL20	0.31	IL1RT2†	0.54
TNFRSF11A*†	0.82	CXCL1	0.76	CASP8	0.38	TRANCE	0.59	HGF	0.30	HGF*†	0.53
TNFR2†	0.77	CASP8	0.61	CCL11	0.35	CXCL10	0.37	CSF1	0.26	TNFSF13B†	0.50
PDL1†	0.74	CD40	0.46	HGF	0.35	TNFSF13B	0.34	IL10	0.22	LAPTGFb1	0.48
IL10RB*†	0.73	LAPTGFb1	0.42	CCL4	0.33	CCL3	0.33	CXCL10	0.20	IL18R1†	0.39
IL2RA	0.71	CCL3	0.32	IL10	0.31	CCL4	0.31	LAPTGFb1	0.19	IL17RA	0.35
MMP3	0.60	IL18	0.26	IL8	0.31	CD8A	0.31	CD8A	0.18	CSF1	0.34
CD4†	0.55	IL17RA	0.25	MMP3	0.30	CD4	0.31	CD40	0.17	ITGB2	0.27

Corr. coeff. = correlation coefficients between the proteins and the respective principal components (represents the variance in the protein explained by the principal component).

*Biomarkers of PC1 and PC6 overlapping with the top 10 proteins of the turquoise and yellow cluster, respectively

†Biomarkers of PC1 and PC6 overlapping with the top 10 proteins of PC1 and PC5 in the Northwestern-HFpEF validation cohort, respectively.

Abbreviations: PC = principal component. For abbreviations of biomarkers, see Supplementary Table 1.

Supplementary Table IV. Association between comorbidity burden and cardiac structure/function in PROMIS-HFpEF

Echocardiographic parameter (dependent variable)	N	Model 1		Model 2	
		β-coefficient (95% CI)	P-value	β-coefficient (95% CI)	P-value
LV mass index	226	0.12 (-0.01 to 0.25)	0.07	0.059 (-0.068 to 0.19)	0.37
Relative wall thickness	226	0.16 (0.04 to 0.28)	0.01	0.13 (0.0014 to 0.26)	0.049‡
Mitral E velocity	226	0.18 (0.05 to 0.31)	0.006	0.21 (0.076 to 0.35)	0.002†
e’ velocity	222	-0.0068 (-0.14 to 0.12)	0.91	-0.14 (-0.25 to -0.02)	0.02*†
E/e’ ratio	222	0.082 (-0.05 to 0.21)	0.23	0.16 (0.02 to 0.29)	0.02*†
LA reservoir strain	221	-0.23 (-0.34 to -0.12)	<0.001	-0.1 (-0.20 to -0.002)	0.047‡
LA volume	221	0.22 (0.10 to 0.34)	<0.001	0.13 (0.01 to 0.25)	0.03*†
LA volume index	225	0.069 (-0.05 to 0.19)	0.26	0.02 (-0.10 to 0.15)	0.74*
LV global longitudinal strain	212	-0.21 (-0.34 to -0.080)	0.002	-0.17 (-0.30 to 0.039)	0.01†
RV free wall strain	221	-0.30 (-0.42 to -0.17)	<0.001	-0.31 (-0.44 to -0.18)	<0.001†
TAPSE	223	0.25 (0.12 to 0.38)	<0.001	0.19 (0.063 to 0.32)	0.004†
RV S’ velocity	225	-0.25 (-0.33 to -0.09)	<0.001	-0.26 (-0.38 to -0.13)	<0.001†
TR velocity	190	0.16 (0.017 to 0.30)	0.03	0.15 (0.0027 to 0.30)	0.048‡

All β-coefficients were standardized. Model 1 adjusted for study site. Model 2 adjusted for study site, age, sex, and glomerular filtration rate.

*Additionally adjusted for atrial fibrillation at the time of echocardiography.

†P<0.05 after correction for false discovery rate.

‡P<0.10 after correction for false discovery rate.

CI = confidence interval; LV = left ventricular; LA = left atrial; RV = right ventricular; TAPSE = tricuspid annular plane systolic excursion; TR= tricuspid regurgitation.

Supplementary Table V. Association between comorbidity burden and inflammation principal components/clusters in PROMIS-HFpEF

Inflammation protein PC/cluster	**Model 1**		**Model 2**		**Model 3**	
	β-coefficient (95% CI)	**P-value**	**β-coefficient (95% CI)**	**P-value**	**β-coefficient (95% CI)**	**P-value**
PC1	0.28 (0.15 to 0.41)	<0.001*	0.18 (0.08 to 0.28)	<0.001*	0.18 (0.08 to 0.29)	<0.001*
PC2	0.04 (-0.08 to 0.16)	0.53	0.04 (-0.08 to 0.17)	0.51	0.05 (-0.08 to 0.29)	0.47
PC3	0.16 (0.03 to 0.29)	0.02*	0.14 (0.01 to 0.26)	0.03*	0.13 (0.003 to 0.27)	0.046†
PC4	-0.02 (-0.15 to 0.10)	0.71	-0.01 (-0.14 to 0.12)	0.88	-0.02 (-0.15 to 0.11)	0.80
PC5	0.19 (0.06 to 0.32)	0.004*	0.17 (0.04 to 0.31)	0.01*	0.17 (0.03 to 0.30)	0.01*
PC6	0.22 (0.09 to 0.34)	0.001*	0.22 (0.09 to 0.35)	0.001*	0.21 (0.08 to 0.34)	0.002*
Turquoise	0.31 (0.18 to 0.43)	<0.001*	0.21 (0.12 to 0.31)	<0.001*	0.21 (0.11 to 0.31)	<0.001*
Yellow	0.22 (0.10 to 0.35)	<0.001*	0.20 (0.08 to 0.32)	0.001*	0.19 (0.07 to 0.31)	0.002*
Red	0.09 (-0.04 to 0.22)	0.18	0.03 (-0.10 to 0.15)	0.64	0.04 (-0.09 to 0.16)	0.59

All β-coefficients were standardized. Model 1 adjusted for study site. Model 2 adjusted for study site, age, sex, and glomerular filtration rate. Model 3 adjusted for study site, age, sex, and glomerular filtration rate, and history of autoimmune disease or malignancy.

*P<0.05 after correction for false discovery rate.

†P<0.10 after correction for false discovery rate.

Abbreviations: PC = principal component.

Supplementary Table VI. Association between inflammation principal components/clusters and cardiac structure/function in PROMIS-HFpEF

Echo parameter	*Principal Components*										*Protein Clusters*					
	PC1		PC3		PC4		PC5		PC6		Turquoise		Yellow		Red	
	β-coef	P	β-coef	P	β-coef	P	β-coef	P	β-coef	P	β-coef	P	β-coef	P	β-coef	P
LVMI	-0.007	0.93	-0.072	0.26	-0.077	0.24	0.11	0.09	-0.018	0.78	-0.054	0.52	-0.025	0.72	0.0043	0.95
RWT	**-0.18**	**0.03**	0.0054	0.93	-0.12	0.06	0.043	0.50	-0.016	0.81	-0.2	**0.02**	-0.018	0.80	-0.085	0.21
mitral E	**0.35**	**<0.001****	-0.016	0.82	**0.31**	**<0.001****	**0.14**	**0.04***	0.21	**0.002****	**0.39**	**<0.001****	**0.34**	**<0.001****	**0.2**	**0.007‡**
e' (avg)	-0.0026	0.97	0.0041	0.94	-0.0091	0.88	0.078	0.18	0.094	0.11	0.058	0.42	0.082	0.19	-0.029	0.63
E/e' (avg)	**0.24**	**0.004****	-0.047	0.49	**0.22**	**0.001****	0.086	0.21	0.047	0.50	0.21	**0.02**	0.2	**0.006****	**0.15**	**0.04**
LA strain†	0.11	0.08	-0.033	0.50	-0.024	0.63	-0.048	0.33	-0.01	0.84	0.093	0.13	-0.1	0.06	0.034	0.50
LA volume	0.00088	0.99	0.033	0.58	0.025	0.68	0.054	0.36	-0.087	0.15	0.01	0.89	-0.067	0.29	-0.042	0.50
LAVI	-0.033	0.67	0.044	0.48	0.017	0.79	0.042	0.50	-0.067	0.28	-0.019	0.81	-0.024	0.71	-0.036	0.58
GLS	0.16	0.06	-0.08	0.24	0.033	0.63	-0.065	0.34	-0.11	0.10	0.12	0.18	-0.096	0.19	0.1	0.16
RV strain	0.13	0.12	-0.064	0.35	-0.057	0.41	**-0.15**	**0.03**	-0.16	**0.02**	0.028	0.74	-0.14	0.06	0.055	0.44
TAPSE	0.094	0.27	**-0.13**	**0.05**	0.025	0.71	**-0.13**	**0.06**	-0.2	**0.003****	-0.016	0.84	**-0.19**	**0.01***	-0.012	0.86
RV S'	0.044	0.59	<0.001	0.99	-0.056	0.40	-0.1	0.12	-0.071	0.28	0.027	0.74	-0.083	0.24	0.017	0.80
TR-velocity	**0.23**	**0.02**	-0.04	0.57	-0.039	0.62	**0.21**	**0.007‡**	0.29	**<0.001****	**0.24**	**0.02**	**0.27**	**<0.001****	0.093	0.25

*P-value no longer significant (>0.05) after additional adjustment for history of autoimmune disease or malignancy.

†LA reservoir strain

‡P<0.10 after correction for false discovery rate.

**P<0.05 after correction for false discovery rate.

β coef = β coefficient, standardized; P = P-value.

LVMI = left ventricular mass index; RWT = left ventricular relative wall thickness; avg = average between left ventricular septal and lateral wall; LA=left atrial; LAVI = left atrial volume; GLS = LV global longitudinal strain; RV = right ventricular; TAPSE = tricuspid annular systolic excursion; TR= tricuspid velocity.

Supplementary Table VII. Inflammation as a mediator between comorbidity score and cardiac structure/function in PROMIS-HFpEF

Mediator	**Total effect**			**Direct effect**			**Indirect effect = mediated**			
	Effect (β)	**95% CI**	**P-value**	**Effect (β)**	**95% CI**	**P-value**	**Effect (β)**	**95% CI**	**P-value**	**% mediated**
Outcome = Mitral E velocity										
PC1	3.90	1.46 to 6.19	0.002	2.85	0.61 to 5.32	0.02	1.05	0.40 to 2.16	**0.002*†**	28%
PC5	3.90	1.39 to 6.05	0.002	3.53	1.02 to 5.71	0.006	0.37	-0.08 to 1.20	0.14	—
PC6	3.90	1.58 to 6.27	0.004	3.18	0.54 to 5.51	0.01	0.72	0.12 to 1.89	**0.02*†**	19%
Turquoise	3.90	1.42 to 6.07	<0.001	2.54	0.21 to 4.88	0.046	1.36	0.61 to 2.44	**<0.001*†**	35%
Yellow	3.90	1.54 to 6.32	0.002	2.75	0.36 to 5.13	0.02	1.15	0.46 to 2.22	**<0.001*†**	30%
Outcome = E/e' ratio										
PC1	0.58	0.13 to 1.07	0.02	0.41	-0.04 to 0.90	0.08	0.17	0.05 to 0.35	**0.006*†**	29%
Turquoise	0.56	0.09 to 1.08	0.02	0.41	-0.03 to 0.90	0.08	0.15	0.004 to 0.33	**0.049‡**	26%
Yellow	0.56	0.10 to 1.04	0.02	0.46	0.03 to 0.92	0.04	0.10	0.01 to 0.37	**0.046‡**	18%
Outcome = TR-velocity										
PC1	5.15	-0.41 to 11.37	0.07	3.99	-1.57 to 9.97	0.18	1.16	0.23 to 2.79	**0.02*†**	23%
PC5	5.15	-0.34 to 11.22	0.07	3.47	-1.87 to 9.34	0.22	1.68	-0.02 to 4.45	0.08‡	—
PC6	5.15	-0.51 to 10.88	0.08	3.06	-2.10 to 8.85	0.27	2.10	0.54 to 4.52	**0.01*†**	41%
Turquoise	5.15	-0.91 to 10.71	0.09	3.75	-2.20 to 9.79	0.23	1.40	0.18 to 3.32	**0.04*‡**	27%
Yellow	5.15	-0.59 to 10.80	0.08	3.19	-2.47 to 8.32	0.24	1.97	0.53 to 4.03	**<0.001*†**	38%
Outcome = RV free wall strain										
PC5	-0.89	-1.37 to -0.46	<0.001	-0.82	-1.27 to -0.38	<0.001	-0.07	-0.21 to 0.01	0.15	—
PC6	-0.89	-1.37 to -0.42	<0.001	-0.81	-1.33 to -0.36	<0.001	-0.08	-0.23 to 0.04	0.17	—
Yellow	-0.89	-1.31 to -0.41	<0.001	-0.83	-1.27 to -0.35	0.006	-0.05	-0.18 to 0.03	0.24	—
Outcome = TAPSE										
PC5	-0.06	-0.10 to -0.03	<0.001	-0.06	-0.09 to -0.03	<0.001	-0.004	-0.01 to 0.00	0.22	—
PC6	-0.06	-0.09 to -0.03	<0.001	-0.05	-0.08 to -0.02	<0.001	-0.008	-0.02 to 0.00	**0.03*‡**	13%
Yellow	-0.06	-0.09 to -0.03	<0.001	-0.06	-0.08 to -0.02	<0.001	-0.007	-0.02 to 0.00	0.06‡	—

Mediators represent the principal component scores for PC's and the cluster eigengenes for clusters (represented by cluster color), respectively.

*P<0.05 after additional adjustment for autoimmune disease and malignancy.

†P<0.05 after correction for false discovery rate.

‡P<0.10 after correction for false discovery rate.

β = β coefficient; CI = confidence interval; TR = tricuspid regurgitation; RV = right ventricular; TAPSE = tricuspid annular plane systolic excursion.

Supplementary Table VIII. Mediation analysis in PROMIS-HFpEF after exclusion of patients with heart failure and mid-range ejection fraction (EF < 50%)

		PROMIS-HFpEF cohort - total (N=228)		**PROMIS-HFpEF cohort excluding LVEF < 50%** (N=205)	
Echocardiographic parameter	**Mediator**	**P-value***	**% mediated**	**P-value**	**% mediated**
Mitral E velocity	PC1	0.002	28%	0.008	26%
	PC6	0.02	19%	0.03	15%
	Turquoise cluster	<0.001	35%	0.004	32%
	Yellow cluster	<0.001	30%	<0.001	23%
E/e’ ratio	PC1	0.006	29%	0.01	21%
	Turquoise cluster	0.049	26%	<0.001	20%
	Yellow cluster	0.046	18%	0.006	12%
TR velocity	PC1	0.02	23%	0.008	24%
	PC6	0.01	41%	0.09	33%
	Turquoise cluster	0.04	27%	0.08	27%
	Yellow cluster	<0.001	38%	0.08	31%
TAPSE	PC6	0.03	13%	<0.001	8%

*P-value for the indirect effect; i.e. the mediated effect.

Abbreviations: LVEF = left ventricular ejection fraction; PC = principal component; TR = tricuspid regurgitation; TAPSE = tricuspid annular plane systolic excursion

Supplementary Table IX: Analysis of selected proteins as mediators of the association between comorbidity burden and echocardiographic hemodynamic parameters in PROMIS-HFpEF.

Protein	Mitral E				E/e' ratio				TR-velocity			
	Total effect	Direct effect	Mediation effect	% Mediated*	Total effect	Direct effect	Mediation effect	% Mediated*	Total effect	Direct effect	Mediation effect	% Mediated*
IGFBP7	3.90 (1.51 to 6.11), P=0.002	2.12 (-0.20 to 4.48), P=0.08	1.78 (0.77 to 3.31), P<0.001	46%	0.58 (0.14 to 1.05), P=0.02	0.39 (-0.06 to 0.82), P=0.09	0.19 (0.05 to 0.48), P=0.004	33%	5.15 (-0.79 to 10.64), P=0.09	2.11 (-3.54 to 7.62), P=0.49	3.04 (1.20 to 6.82), P<0.001	59%
GDF15	3.90 (1.69 to 6.23), P<0.001	2.28 (-0.26 to 4.76), P=0.08	1.62 (0.67 to 3.30), P<0.001	42%	0.58 (0.14 to 1.08), P=0.02	0.42 (-0.04 to 0.90), P=0.07	0.16 (0.0003 to 0.41), P=0.08	27%	5.15 (-0.35 to 10.94), P=0.07	1.84 (-3.61 to 7.33), P=0.52	3.31 (1.04 to 6.67), P=0.004	64%
TNFR1	1.23 (0.43 to 2.48), P<0.001	2.67 (0.10 to 4.72), P=0.04	1.23 (0.43 to 2.48), P<0.001	32%	0.58 (0.11 to 1.04), P=0.01	0.38 (-0.10 to 0.87), P=0.16	0.20 (0.03 to 0.38), P=0.02	34%	5.15 (-0.12 to 11.81), P=0.07	3.12 (-2.49 to 9.08), P=0.27	2.03 (0.50 to 4.29), P=0.02	39%
UPAR	3.90 (1.64 to 6.13), P=0.002	3.03 (0.55 to 5.45), P=0.02	0.87 (0.32 to 2.42), P=0.002	22%	0.58 (0.17 to 1.10), P=0.01	0.48 (0.06 to 0.94), P=0.03	0.11 (0.02 to 0.30), P=0.04	18%	5.15 (-0.67 to 10.71), P=0.07	3.68 (-2.05 to 9.15), P=0.18	1.47 (0.40 to 3.37), P=0.02	29%
LTBR	3.90 (1.32 to 5.86), P=0.002	3.03 (0.35 to 4.94), P=0.01	0.87 (0.27 to 1.78), P=0.002	22%	0.58 (0.14 to 1.11), P=0.02	0.51 (0.04 to 1.03), P=0.04	0.07 (-0.02 to 0.20), P=0.10	—	5.15 (-0.46 to 10.72), P=0.08	4.15 (1.15 to 9.57), P=0.15	1.001 (0.20 to 2.56), P=0.02	19%
IL1RT1	3.90 (1.45 to 6.19), P<0.001	3.35 (0.66 to 5.64), P=0.008	0.55 (0.07 to 1.52), P=0.04	14%	0.58 (0.15 to 1.03), P=0.02	0.56 (0.13 to 1.03), P=0.02	0.02 (-0.03 to 0.13), P=0.47	—	5.15 (-0.86 to 10.96), P=0.09	3.54 (-2.07 to 8.80), P=0.23	1.60 (0.23 to 4.10), P=0.03	31%
PLC	3.90 (1.79 to 6.10), P<0.001	2.25 (-0.34 to 4.54), P=0.10	1.65 (0.56 to 3.05), P<0.001	42%	0.58 (0.18 to 1.09), P=0.01	0.41 (-0.010 to 0.90), P=0.08	0.17 (-0.01 to 0.41), P=0.08	—	5.15 (-0.90 to 11.05), P=0.09	4.93 (-1.65 to 11.05), P=0.08	0.22 (-1.40 to 2.27), P=0.82	—

Protein	Mitral E				E/e’ ratio				TR-velocity			
	Total effect	**Direct effect**	**Mediation effect**	**% Mediated ***	**Total effect**	**Direct effect**	**Mediation effect**	**% Mediated ***	**Total effect**	**Direct effect**	**Mediation effect**	**% Mediated***
TNFSF14	3.90 (1.74 to 6.17), P<0.001	4.00 (1.73 to 6.33), P<0.001	-0.10 (-0.56 to 0.11), P=0.49	—	0.58 (0.14 to 1.08), P=0.004	0.60 (0.17 to 1.09), P=0.002	-0.02 (-0.12 to 0.03), P=0.56	—	5.15 (-0.40 to 11.04), P =0.07	5.30 (-0.19 to 11.05), P=0.06	-0.15 (-1.92 to 0.12), P=0.58	—
Notch3	3.90 (1.45 to 6.17), P=0.002	3.77 (1.52 to 5.93), 0.002	0.13 (-0.33 to 0.61), P=0.55	—	0.58 (0.14 to 1.09), P=0.02	0.58 (0.15 to 1.11), P=0.02	-0.0004 (-0.05 to 0.06), P=0.97	—	5.15 (-0.20 to 11.43), P=0.07	4.71 (-0.32 to 11.09), P=0.10	0.44 (-0.08 to 1.45), P=0.18	—
VEGFA	3.90 (1.71 to 6.28), P<0.001	3.17 (0.70 to 5.68), P=0.004	0.73 (-0.10 to 1.86), P=0.12	—	0.58 (0.12 to 1.09), P=0.02	0.54 (0.09 to 1.07), P=0.04	0.04 (-0.12 to 0.18), P=0.65	—	5.15 (-0.76 to 11.16), P=0.09	4.84 (-1.30 to 10.92), P=0.13	0.31 (-1.05 to 1.88), P=0.71	—
FABP4	3.90 (1.81 to 6.08), P<0.001	3.71 (1.13 to 6.22), P<0.001	0.18 (-1.13 to 1.54), P=0.72	—	0.58 (0.13 to 1.08), P=0.01	0.72 (0.26 to 1.21), P=0.004	-0.14 (-0.34 to 0.10), P=0.18	—	5.15 (-0.90 to 11.11), P=0.10	5.23 (-1.54 to 11.61), P=0,14	-0.08 (-2.15 to 2.00), P=0,97	—
IL6	3.90 (1.67 to 6.19), P<0.001	3.39 (1.10 to 5.66), P=0.004	0.50 (-0.03 to 1.38), P=0.12	—	0.58 (0.14 to 1.07), P=0.02	0.51 (0.02 to 1.04), P=0.05	0.07 (-0.04 to 0.22), P=0.19	—	5.15 (-0.94 to 11.57), P=0.10	4.37 (-1.53 to 10.37), P=0.12	0.78 (-0.20 to 2.99), P=0.22	—
ICAM2	3.90 (1.56 to 6.30), P=0.002	3.80 (1.42 to 6.23), P=0.002	0.10 (-0.07 to 0.62), P=0.41	—	0.58 (0.11 to 1.08), P=0.02	0.58 (0.11 to 1.08), P=0.02	0.001 (-0.05 to 0.06), P=0.98	—	5.15 (0.02 to 10.91), P=0.06	5.12 (-0.06 to 10.84), P=0.06	0.04 (-0.66 to 0.73), P=0.93	—
IL10RB	3.90 (1.46 to 6.19), P<0.001	3.44 (0.98 to 5.68), P=0.008	0.46 (-0.12 to 1.37), P=0.14	—	0.58 (0.14 to 1.03), P=0.008	0.54 (0.08 to 1.00), P=0.03	0.04 (-0.01 to 0.17) P=0.24	—	5.15 (-0.75 to 10.87), P=0.09	4.71 (-1.23 to 10.50), P=0.12	0.44 (-0.04 to 1.80), P=0.16	—
MCP1	3.90 (1.63 to 6.11), P<0.001	3.87 (1.59 to 6.10), P<0.001	0.03 (-0.15 to 0.53), P=0.84	—	0.58 (0.12 to 1.08), P=0.01	0.58 (0.12 to 1.09), P=0.02	-0.002 (-0.08 to 0.04), P=0.94	—	5.15 (-0.59 to 10.74), P=0.09	5.02 (-0.55 to 10.73), P=0.09	0.14 (-0.51 to 0.71), P=0.71	—

Values for total, direct and indirect effect indicate the β coefficient (95% confidence interval), P-value.
* Only provided when the mediated effect was significant.
Abbreviations: TR = tricuspid regurgitation; for abbreviations of biomarkers, see Supplementary Table 1.

Supplementary Table X. Association between systemic inflammation and measures of coronary microvascular disease, peripheral endothelial function, and exercise capacity

	Coronary flow reserve				***Reactive hyperemia index***				***6MWT distance***			
Inflammation protein PC/cluster	**Minimally-adjusted**		**Multivariable-adjusted**		**Minimally-adjusted**		**Multivariable-adjusted**		**Minimally-adjusted**		**Multivariable-adjusted**	
	β-coef	P-value	β-coef	P-value	β-coef	P-value	β-coef	P-value	β-coef	P-value	β-coef	P-value
PC1	-0.12	0.10	-0.11	0.26	-0.043	0.43	-0.035	0.48	-0.29	***<0.001***	-0.35	***<0.001***
PC2	-0.12	0.14	-0.10	0.20	-0.052	0.38	-0.052	0.37	-0.093	0.19	-0.079	0.24
PC3	-0.063	0.40	-0.072	0.34	-0.072	0.20	-0.083	0.31	-0.15	***0.02***	-0.19	***0.002***
PC4	-0.024	0.75	-0.064	0.41	-0.044	0.44	-0.046	0.42	-0.031	0.66	0.011	0.88
PC5	-0.22	***0.003***	-0.19	***0.01***†	-0.075	0.18	-0.068	0.40	-0.16	***0.02***	-0.19	***0.003***
PC6	-0.2	***0.009***	-0.18	***0.02****	-0.008	0.88	0.0047	0.92	-0.24	***<0.001***	-0.19	***0.003***
Turquoise	-0.12	0.10	-0.15	0.14	-0.045	0.41	-0.046	0.40	-0.33	***<0.001***	-0.42	***<0.001***
Yellow	-0.21	***0.007***	-0.18	***0.03***	-0.036	0.53	-0.013	0.76	-0.33	***<0.001***	-0.27	***<0.001***
Red	-0.12	0.09	-0.10	0.18	-0.083	0.13	-0.080	0.35	-0.13	***0.04***	-0.078	0.25

Minimally-adjusted models: covariate = study site.

Multivariable-adjusted models: covariates = age, gender, GFR, and study site.

*P-value <0.05 after additional adjustment for smoking, history of coronary artery disease, and E/e' ratio.

†P-value <0.01 after additional adjustment for smoking, history of coronary artery disease, and E/e' ratio.

Abbreviations: β-coef = beta-coefficient; reactive hyperemia index = marker of peripheral endothelial function measured by EndoPAT; 6MWT = six minute walk test; PC = principal component.

Supplementary Table XI: Comparison of characteristics of HFpEF patients versus (1) controls in the Northwestern University validation cohort, and (2) HFpEF patients in the PROMIS-HFpEF cohort

Characteristic	Northwestern University cohort			PROMIS-HFpEF	
	Controls (n=30)	HFpEF patients (n=117)	P-value†	HFpEF patients (n=228)	P-value‡
Age, years	67 (58-73)	70 (61-78)	0.16	75 (70-81)	<0.001
Female, n(%)	10 (33.3)	76 (65.0)	0.003	129 (56.3)	0.13
Race, n(%) -White -African-American -Other	 15 (88.2) 0 (0) 2 (11.8)	 59 (80.8) 7 (9.6) 7 (9.6)	0.58	 200 (87.3) 10 (4.4) 19 (8.3)	0.22
BMI, kg/m^2	27.1 (24.6-32.7)	32.2 (26.5-41.6)	0.001	28.1 (24.4-32.4)	<0.001
Obesity (BMI ≥30 kg/m^2), n(%)*	11 (40.7)	72 (61.5)	0.06	85 (37.1)	<0.001
Heart rate, bpm	76 (60-78)	70 (62-76)	0.51	69 (61-79)	0.97
Systolic BP, mmHg	126 (114-138)	124 (112-133)	0.48	139 (125-152)	<0.001
Diastolic BP, mmHg	73 (66-80)	70 (61-80)	0.29	77 (69-85)	<0.001
Hypertension, n(%)*	19 (67.9)	91 (78.4)	0.32	190 (83.0)	0.31
Hyperlipidemia, n(%)*	16 (57.1)	72 (62.1)	0.67	121 (52.8)	0.11
Diabetes mellitus, n(%)*	5 (17.9)	44 (37.9)	0.048	63 (27.5)	0.05
Coronary artery disease, n(%)*	19 (67.9)	51 (44.0)	0.03	48 (21.0)	<0.001
Stroke, n(%)*	1 (3.6)	9 (7.8)	0.69	26 (11.4)	0.35
Anemia, n(%)*	4 (14.8)	46 (39.7)	0.02	79 (34.5)	0.41
Comorbidity score	3 (2-4)	4 (2-4)	0.50	3 (2-4)	0.83
Atrial fibrillation, n(%)*	20 (66.7)	54 (46.2)	0.07	131 (57.2)	0.05
Sodium, mmol/L	138 (137-139)	139 (137-140)	0.25	140 (138-142)	<0.001
Potassium, mmol/L	3.9 (3.7-4.2)	4.0 (3.7-4.4)	0.40	4.2 (3.9-4.5)	0.001
BUN, mg/dL	16.0 (12.0-19.1)	21.6 (15.1-28.0)	0.001	22.4 (17.1-26.7)	0.37
Creatinine, mg/dL	1.06 (0.85-1.23)	1.19 (0.97-1.60)	0.008	1.05 (0.86-1.33)	0.93
GFR, mL/min/1.73 m^2	61 (59-61)	59 (45-61)	0.003	59 (46-73)	<0.001
BNP, pg/ml	44 (18-81)	120 (45-269)	0.08	—	—
NT-proBNP, pg/ml	—	—	—	1480 (827-2518)	—
Hemoglobin, g/dL	14.5 (12.4-15.0)	12.7 (11.3-13.7)	<0.001	13.0 (11.9-14.0)	0.04
White blood cell count, K/mm^3	6.7 (5.7-8.3)	6.8 (5.4-8.3)	0.85	6.6 (5.5-7.8)	0.36
Platelet count, K/mm^3	231 (181-300)	230 (192-272)	0.79	221 (182-252)	0.16
ACE-inhibitor	6 (22.2)	26 (22.4)	1.0	72 (31.4)	0.10
ARB	7 (25.9)	22 (19.0)	0.43	100 (43.7)	<0.001
Beta-blocker	13 (48.1)	69 (60.0)	0.29	176 (76.9)	0.002
MRA	0 (0)	54 (46.6)	<0.001	63 (27.5)	0.001
Loop diuretic	1 (3.7)	83 (71.6)	<0.001	125 (54.6)	0.002
Thiazide diuretic	9 (33.3)	16 (13.8)	0.02	26 (11.4)	0.60
Statin	21 (77.8)	67 (57.8)	0.08	131 (57.2)	1.0
Antiplatelet agent	17 (63.0)	68 (58.6)	0.83	59 (25.8)	<0.001
LV ejection fraction, %	64 (61-70)	64 (58-69)	0.29	59 (55-64)	<0.001
LV end-diastolic volume index, ml/m^2	37.3 (32.8-46.1)	35.1 (29.1-43.3)	0.78	41.2 (33.4-50.1)	<0.001

Characteristic	Northwestern University cohort			PROMIS-HFpEF	
	Controls (n=30)	HFpEF patients (n=117)	P-value†	HFpEF patients (n=228)	P-value‡
LV end-systolic volume index, ml/m^2	13.2 (10.2-17.3)	13.1 (10.1-16.6)	0.23	16.7 (12.6-21.1)	<0.001
LV mass index, g/m^2	85 (68-110)	85 (69-105)	0.70	103 (84-125)	<0.001
LV mass, g	165 (130-228)	184 (138-217)	0.43	198 (162-245)	0.03
Relative wall thickness	0.47 (0.40-0.54)	0.48 (0.39-0.54)	0.76	0.45 (0.39-0.52)	0.27
LA volume index, ml/m^2	23.0 (17.3-27.5)	26.0 (20.1-34.1)	0.04	38.4 (30.7-45.1)	<0.001
LA volume, ml	40.8 (33.1-55.0)	52.5 (41.5-71.0)	0.09	74.0 (57.5-89.3)	<0.001
Mitral E velocity, cm/s	74 (58-94)	95 (74-117)	0.01	94 (80-115)	0.95
e' (average), cm/s	7.6 (6.3-9.8)	7.7 (6.0-9.3)	0.75	8.2 (6.6-10.0)	0.03
E/e' ratio (average)	10.2 (7.1-12.4)	12.3 (9.1-16.0)	0.08	12.2 (9.2-16.2)	1.0
RVEDA index, cm^2/m^2	89 (71-107)	85 (71-99)	0.52	97 (82-113)	<0.001
RVESA index, cm^2/m^2	51 (42-70)	49 (40-59)	0.61	56 (47-71)	<0.001
RV wall thickness, cm	0.46 (0.39-0.50)	0.55 (0.50-0.66)	0.009	0.48 (0.44-0.54)	<0.001
TAPSE, cm	1.9 (1.7-2.1)	1.9 (1.4-2.2)	0.78	1.8 (1.6-2.1)	0.39
TR velocity, cm/s	210 (177-230)	288 (246-325)	<0.001	297 (267-329)	<0.001

Values depict median (25th-75th percentile) for continuous variables and frequency (percentage) for categorical variables.

*Comorbidities included in the comorbidity score

†Comparing HFpEF patients versus controls in the Northwestern University validation cohort

‡Comparing HFpEF patients from the PROMIS study (derivation cohort) with HFpEF patients from the Northwestern University cohort (validation cohort)

Abbreviations: BMI = body mass index; BP = blood pressure; HF = heart failure; BUN = blood urea nitrogen; BNP = B-type natriuretic peptide; NT-proBNP = N-terminal pro-B-type natriuretic peptide; GFR = glomerular filtration rate; ACE = angiotensinogen converting enzyme; ARB = angiotensin II receptor blocker; MRA = mineralocorticoid receptor antagonist; LV = left ventricular; LA = left atrial; RV = right ventricular; RVEDA = right ventricular end-diastolic area; RVESA = right ventricular end-systolic area; TAPSE = tricuspid annular plane systolic excursion; TR = tricuspid regurgitation

Supplementary Table XII. Association between comorbidity burden and systemic inflammation in the Northwestern HFpEF patients

	Unadjusted		**Adjusted***
Dependent variable	**Standardized β-coefficient (95% CI)**	**P-value**	**P-value**
PC1	0.21 (0.04 to 0.39)	**0.02**	0.37
PC2	0.14 (-0.04 to 0.32)	0.14	0.17
PC3	-0.07 (-0.26 to 0.11)	0.44	0.36
PC4	0.11 (-0.06 to 0.29)	0.23	0.44
PC5	-0.03 (-0.21 to 0.15)	0.75	0.33
PC6	-0.09 (-0.28 to 0.10)	0.33	0.38
Red	0.08 (-0.10 to 0.27)	0.37	0.51
Yellow	0.17 (-0.007 to 0.35)	0.06	0.67
Turquoise	0.23 (0.05 to 0.41)	**0.01**	**0.049**

*Adjusted for age, gender and GFR

Abbreviations: PC = principal component.

Supplementary Table XIIIA. Unadjusted associations between inflammation PCs/clusters and cardiac structure/function in the Northwestern HFpEF patients

Dependent variable	**PC1**		**PC2**		**PC3**		**PC4**		**PC5**		**PC6**		**Turquoise**	
	β-coef	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**
LVMI	0.17	*0.08*	-0.07	0.47	0.07	0.47	-0.19	*0.05*	-0.002	0.98	0.05	0.60	0.13	0.32
RWT	0.06	0.53	0.02	0.85	-0.05	0.63	0.11	0.26	-0.14	0.14	0.09	0.37	0.05	0.71
Mitral E	0.28	***0.003***	-0.16	0.11	0.07	0.45	0.006	0.95	0.09	0.34	-0.01	0.91	0.22	*0.09*
e’ (avg)	-0.03	0.76	0.16	0.10	0.01	0.88	0.09	0.33	-0.02	0.81	0.09	0.38	-0.10	0.47
E/e’ (avg)	0.19	*0.06*	-0.15	0.13	0.03	0.78	-0.03	0.72	0.05	0.61	-0.03	0.74	0.21	0.12
LA volume	0.22	***0.02***	-0.16	*0.09*	-0.05	0.57	-0.16	*0.09*	0.15	0.13	0.009	0.93	0.14	0.28
LAVI	0.17	*0.08*	-0.30	***0.002***	-0.10	0.29	-0.19	***0.046***	0.10	0.30	0.01	0.88	0.09	0.50
TAPSE	-0.07	0.46	0.10	0.32	0.12	0.23	-0.13	0.18	-0.11	0.25	0.07	0.47	0.02	0.90
TR-velocity	0.12	0.26	-0.21	***0.04***	0.04	0.72	0.07	0.52	0.15	0.15	0.04	0.71	0.12	0.41

β-coef = beta coefficient, standardized

PC1-PC6 = principal components of inflammatory circulating biomarkers; Turquoise, Yellow and Red = clusters of inflammatory biomarkers following network analysis.

Abbreviations: LVMI = left ventricular mass index; RWT = left ventricular relative wall thickness; avg = average between left ventricular septal and lateral walls; LA=left atrial; LAVI = left atrial volume; TAPSE = tricuspid annular systolic excursion; TR= tricuspid velocity.

Supplementary Table XIIIB. Multivariable-adjusted associations between inflammation PCs/clusters and cardiac structure/function in the Northwestern HFpEF patients

Dependent variable	**PC1**		**PC2**		**PC3**		**PC4**		**PC5**		**PC6**		**Turquoise**	
	β-coef	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**	**β-coef**	**P-value**
LVMI	0.15	0.28	-0.03	0.77	0.05	0.59	-0.23	***0.02***	-0.03	0.78	0.08	0.41	0.13	0.32
RWT	0.12	0.39	0.18	*0.09*	-0.06	0.50	0.11	0.25	-0.13	0.17	0.10	0.30	0.05	0.71
Mitral E	0.31	***0.03***	-0.14	0.19	0.05	0.61	-0.005	0.96	0.06	0.54	-0.02	0.81	0.22	*0.09*
e’ (avg)	-0.03	0.84	0.06	0.57	0.008	0.93	0.08	0.42	-0.02	0.79	0.13	0.17	-0.08	0.52
E/e’ (avg)	0.24	*0.08*	-0.07	0.52	0.02	0.81	-0.03	0.74	0.04	0.71	-0.05	0.59	0.20	0.12
LA volume	0.13	0.36	-0.16	0.11	-0.06	0.49	-0.25	***0.008***	0.09	0.32	0.04	0.65	0.14	0.28
LAVI	0.07	0.58	-0.21	***0.03***	-0.12	0.18	-0.25	***0.005***	0.07	0.45	0.03	0.71	0.09	0.49
TAPSE	0.06	0.66	-0.04	0.69	0.14	0.14	-0.07	0.46	-0.10	0.29	0.03	0.72	0.02	0.90
TR-velocity	0.07	0.65	-0.18	0.12	0.02	0.85	0.03	0.75	0.14	0.19	0.03	0.74	0.12	0.41

β-coef = beta coefficient, standardized

All regression models were adjusted for age, gender, and GFR; models for LA volume, LAVI, e’, and E/e’ were additionally adjusted for atrial fibrillation.

Abbreviations: LVMI = left ventricular mass index; RWT = left ventricular relative wall thickness; avg = average between left ventricular septal and lateral walls; LA=left atrial; LAVI = left atrial volume; PC = principal component; TAPSE = tricuspid annular systolic excursion; TR= tricuspid velocity.