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CLINICAL RESEARCH

Pathophysiologic Processes and Novel Biomarkers Associated With Congestion in Heart Failure

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ABSTRACT

BACKGROUND Congestion is the main driver behind symptoms of heart failure (HF), but pathophysiology related to congestion remains poorly understood.

OBJECTIVES Using pathway and differential expression analyses, the authors aim to identify biological processes and biomarkers associated with congestion in HF.

METHODS A congestion score (sum of jugular venous pressure, orthopnea, and peripheral edema) was calculated in 1,245 BIOSTAT-CHF patients with acute or worsening HF. Patients with a score ranking in the bottom or top categories of congestion were deemed noncongested (n = 408) and severely congested (n = 142), respectively. Plasma concentrations of 363 unique proteins (Olink Proteomics Multiplex CVD-II, CVD-III, Immune Response and Oncology II panels) were compared between noncongested and severely congested patients. Results were validated in an independent validation cohort of 1,342 HF patients (436 noncongested and 232 severely congested).

RESULTS Differential protein expression analysis showed 107/363 up-regulated and 6/363 down-regulated proteins in patients with congestion compared with those without. FGF-23, FGF-21, CA-125, soluble ST2, GDF-15, FABP4, IL-6, and BNP were the strongest up-regulated proteins (fold change [FC] >1.30, false discovery rate [FDR], $P < 0.05$). KITLG, EGF, and PON3 were the strongest down-regulated proteins (FC <−1.30, FDR $P < 0.05$). Pathways most prominently involved in congestion were related to inflammation, endothelial activation, and response to mechanical stimulus. The validation cohort yielded similar findings.

CONCLUSIONS Severe congestion in HF is mainly associated with inflammation, endothelial activation, and mechanical stress. Whether these pathways play a causal role in the onset or progression of congestion remains to be established. The identified biomarkers may become useful for diagnosing and monitoring congestion status.
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ABBREVIATIONS AND ACRONYMS

BNP = B-type natriuretic peptide

CA-125 = cancer antigen 125

CCS = clinical congestion score

EGF = epidermal growth factor

EGFR = epidermal growth factor receptor

FABP4 = fatty acid–binding protein 4

FC = fold change

FDR = false discovery rate

FGF = fibroblast growth factor

GDF = growth differentiation factor

IL = interleukin

KITLG = Kit ligand

MUC16 = mucin-16

NT-proBNP = N-terminal pro-B-type natriuretic peptide

PON3 = paraoxonase 3

ST2 = suppressor of tumorigenicity 2

Congestion is the main driver for symptoms and hospitalization of patients with heart failure (HF). Although several mechanisms for congestion have been proposed in HF, limited studies have examined their interactions using large-scale clinical cohorts. Traditionally, the main proposed mechanism is that impaired contractility of the heart results in increased intracardiac filling pressures, causing interstitial fluid accumulation.[1,2] Second, reduced cardiac output triggers neurohormonal activation causing additional fluid retention. Other contributory mechanisms have been proposed as well, including inflammation and endothelial cell dysfunction.[3-6] Increased mechanical stress on blood vessels leads to vascular endothelial production of local inflammatory cytokines and reactive oxygen molecules, eventually leading to endothelial dysfunction.[6] Previous studies suggest that peripheral venous congestion may even be a primary source of acute clinical decompensation, triggering a cascade of proinflammatory, oxidative, and hemodynamic stress events.[4-6] However, these pathways have been investigated as isolated components in separate studies, linked to a limited number of proteins/biomarkers, and their interactions in an HF patient remain unclear.

In the present study, we performed a pathway analysis, a well-validated approach, using multiple biomarkers from different pathophysiologic domains to explore pathways that are associated with congestion in patients with new-onset and worsening HF. These findings may help to identify novel diagnostic markers and future therapies to improve congestion in patients with HF.

SEE PAGE 633

METHODS

STUDY DESIGN AND POPULATION. We performed an analysis of the BIOSTAT-CHF (Biology Study to Tailored Treatment in Chronic Heart Failure) population, a multicenter, prospective, observational study. Study design and main results are published elsewhere.[7] The BIOSTAT-CHF index cohort included 2,516 patients from 11 European countries with signs and/or symptoms of new-onset or worsening HF. The BIOSTAT-CHF validation cohort consisted of 1,738 patients from 6 centers in Scotland. Both inpatients and outpatients were included, with the majority of the study patients having worsening HF. For this, the last measured LVEF in the past 24 months and/or B-type natriuretic peptide (BNP)/N-terminal (NT) proBNP plasma levels from outpatient visit or inpatient hospitalization were used. Briefly, for the index cohort, enrollment criteria included left ventricular ejection fraction (LVEF) ≤40% or plasma concentrations of NT-proBNP >2,000 pg/mL and/or BNP >400 pg/mL.[7] At the time of inclusion, patients from the index cohort were treated with ≥40 mg/day intravenous or oral furosemide or equivalent. The validation cohort included HF patients receiving ≥20 mg/day furosemide or equivalent or with a history of previous HF hospitalization. The additional NT-proBNP/BNP criteria were not recommended for the validation cohort. At the time of inclusion, patients from both cohorts were not previously treated with or were receiving ≤50% of European Society of Cardiology guideline-recommended target doses of angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, and/or beta-blockers. During the optimization phase in the first 3 months, evidence-based medications were initiated or up-titrated according to the guidelines. This was followed by a 6-month maintenance phase in which no optimization was predicted, except during changes in clinical status. Blood sample collection was done on the day of inclusion and not at a set time point. Additional information on the study cohort and measurements can be found in previous publications.[8,9] The study adhered to the Declaration of Helsinki and was approved by local and national ethical committees.[7] Written informed consents were obtained for both cohorts.

STUDY ASSESSMENTS. The clinical congestion score (CCS) was defined as the sum of jugular venous pressure (no = 0; yes = 1), orthopnea (no = 0; yes = 1), and peripheral edema (not present = 0; around ankle = 1; below knee = 2; above knee = 3), with a maximum score of 5 in the index cohort. The score was limited to these variables in order to get a sufficient study sample size and improve interpretation, and the focus on extreme groups supports the isolation of biomarkers that differ strongly in expression levels in both groups. This score is a derivation of the EVEREST congestion score[10] and has been validated in previous studies.[11,12] The maximum score was 4 in the validation cohort, because orthopnea was not available. After this, patients were divided into groups of no congestion (score 0 for both cohorts) and severe congestion (scores 4 and 5 for the index cohort and 3 and 4 for the validation cohort). In total, 1,245 patients had available congestion score and biomarker data in the index cohort, and 1,342 patients in the validation cohort. Patients without available

congestion score (n = 992 index; n = 371 validation) or Olink biomarker measurements (n = 277 index; n = 25 validation) were excluded (total excluded: n = 1,271 index; n = 396 validation). In addition, 2 patients with creatinine values >930 μmol/L were excluded from the index cohort, because these values were outliers. For the index cohort, 408 patients and 142 patients were included in bottom and top CCS groups, respectively. For the validation cohort, 436 patients and 232 patients were included (Supplemental Figure 1). Estimated glomerular filtration rate was calculated according to the CKD-EPI formula.

PROTEIN BIOMARKER MEASUREMENTS. The Olink Proteomics Multiplex Cardiovascular Disease (CVD) II, CVD-III, Immune Response and Oncology II panels of 363 unique proteins were used in both the BIOSTAT index and validation cohorts to investigate the biomarker profiles of the 2 study groups. Measurements were performed by Olink Bioscience analysis service (Uppsala, Sweden), using the Proseek Multiplex 96 × 96 kit, which measures human protein biomarkers in 1-μL plasma samples.[13] Olink multiplexing is based on the Proximity Extension Assay (PEA) technology, where oligonucleotide-bound antibody probe pairs bind to their corresponding protein biomarker targets in the sample. The hybridized oligonucleotides are then extended by a DNA polymerase, and the resulting DNA barcode is amplified with the use of polymerase chain reaction. The biomarker quantity is then transformed on a log2 scale to yield a normalized protein expression value. The full list of biomarkers studied is presented in Supplemental Table 1.

STATISTICAL ANALYSIS. Baseline characteristics of congested and noncongested patients were compared with the use of Wilcoxon rank-sum or Kruskal-Wallis test for continuous nonnormally distributed variables, Student's *t*-test or analysis of variance (ANOVA) for continuous normally distributed variables, and chi-square test for categoric variables. The *P* value for trend was computed with the use of Pearson, Spearman, and Mantel-Haenszel tests for continuous normally distributed, nonnormally distributed, and categoric variables, respectively. All analyses were performed with the use of R version 3.6.3 (R Foundation for Statistical Computing). Two-sided tests were used, and $P < 0.05$ was considered to be significant. Baseline tables were created with the use of compareGroups package version 4.4.1.[14]

Differential expression analysis. Differential protein expression of the 363 protein biomarkers between noncongested and severely congested patients at baseline was calculated with the use of the R package Linear models for microarray analysis software (Limma version 3.44.3).[15] We visualized the differences in expression of biomarkers (up-regulation or down-regulation) in both groups with the use of volcano plots. The volcano plots were also adjusted for age and sex. Biomarkers with false discovery rate (FDR)-corrected $P < 0.05$ and minimum fold change (FC) cutoff of 1.30 (or 0.38 in log2 form) were considered to be significant. The significant biomarkers were further used for pathway analyses.

Pathway overrepresentation analyses. We performed pathway overrepresentation analyses for biomarkers at baseline using Cytoscape (version 3.8.0) and ClueGO (version 2.5.7). The following databases were used as reference: Gene Ontology (GO) biological processes, Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome pathways. The pathway networks were constructed using "all-experimental" evidence type, a Bonferroni step-down procedure, and a right-sided hypergeometric test. Only biological processes/pathways with an FDR ≤0.01 are reported.

Outcome analyses. Cox proportional hazard regression analyses were performed in both cohorts for the categories of congestion (bottom, middle, and top) for the outcomes of all-cause mortality and the combined end point of all-cause mortality and first occurrence of HF hospitalization. Multivariable models were adjusted for the variables from the BIOSTAT-CHF model published previously.[16]

RESULTS

BASELINE CHARACTERISTICS. Baseline characteristics of patients without congestion and with severe congestion in the index and validation cohorts are presented in **Table 1** and Supplemental Table 2, respectively. Baseline characteristics of the population across different CCS groups (including the middle group) are presented in Supplemental Tables 3 and 4. In the index cohort, severely congested patients were older, more symptomatic (higher New York Heart Association [NYHA] functional class), and had higher plasma NT-proBNP concentrations. The prevalence of a history of atrial fibrillation, diabetes, and renal disease were higher in more severely congested patients (all $P < 0.002$) (**Table 1**). Similar findings were observed in the validation cohort (Supplemental Table 2). Baseline characteristics of included and excluded population of both cohorts are shown in Supplemental Tables 5 and 6.

DIFFERENTIAL EXPRESSION ANALYSES. In the index cohort, 107/363 proteins were up-regulated, and

TABLE 1 Baseline Table According to Extreme Groups of Bottom and Top Baseline Congestion Score (Index Cohort)

	No Congestion (CCS 0) (n = 408)	Severe Congestion (CCS 4 or 5) (n = 142)	*P* Value
Male	317 (77.7)	106 (74.6)	0.531
Age, y	66.6 ± 11.7	69.6 ± 12.8	0.016[a]
Weight, kg	80.5 ± 16.3	90.1 ± 20.4	<0.001[a]
Height, cm	172 ± 9.01	173 ± 8.47	0.120
BMI, kg/m^2	27.2 ± 4.69	30.0 ± 6.36	<0.001[a]
Heart rate, beats/min	74.6 ± 17.0	84.2 ± 20.3	<0.001[a]
SBP, mm Hg	126 ± 19.2	123 ± 24.3	0.329
DBP, mm Hg	76.2 ± 11.7	73.5 ± 14.5	0.048[a]
LVEF, %	30.7 ± 9.07	31.7 ± 12.5	0.427
Ejection fraction ≥40%	61 (15.6)	28 (25.5)	0.024[a]
Clinical profile			
NYHA functional class			<0.001
I	38 (10.3)	7 (5.98)	
II	235 (63.7)	45 (38.5)	
III	92 (24.9)	57 (48.7)	
IV	4 (1.08)	8 (6.84)	
III/IV	96 (26.0)	65 (55.6)	<0.001[a]
Pulmonary congestion (single/bi-basilar)	110 (27.6)	112 (79.4)	<0.001[a]
JVP (yes)	0 (0.0)	133 (93.7)	<0.001[a]
Orthopnea (yes)	0 (0.0)	126 (88.7)	<0.001[a]
Peripheral edema			<0.001[a]
Not present	408 (100)	0 (0.0)	
Ankle	0 (0.0)	0 (0.0)	
Below knee	0 (0.0)	77 (54.2)	
Above knee	0 (0.0)	65 (45.8)	
Inpatients	204 (50.0)	133 (93.7)	<0.001[a]
Patient history			
Previous HF hospitalization in past year	115 (28.2)	44 (31.0)	0.599
Ischemic heart disease (primary/secondary)	239 (64.6)	69 (56.6)	0.138
Myocardial infarction	166 (40.7)	48 (33.8)	0.177
CABG	69 (16.9)	32 (22.5)	0.172
PCI	91 (22.3)	23 (16.2)	0.154
Atrial fibrillation	153 (37.5)	81 (57.0)	<0.001[a]
Stroke	27 (6.62)	15 (10.6)	0.180
Peripheral artery disease	32 (7.84)	16 (11.3)	0.283
Hypertension	271 (66.4)	81 (57.0)	0.057
ICD/CRT/pacemaker/biventricular pacer	84 (20.6)	45 (31.7)	0.010[a]
Diabetes	106 (26.0)	57 (40.1)	0.002[a]
COPD	52 (12.7)	28 (19.7)	0.059
Renal disease	95 (23.3)	68 (47.9)	<0.001[a]

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6/363 proteins were down-regulated in highly congested compared with noncongested patients (**Figure 1**). In the validation cohort, 54/363 proteins were up-regulated and 2/363 were down-regulated. Both cohorts had 1 down-regulated and 51 up-regulated proteins in common (**Figure 1**). Differentially expressed proteins were consistent in both cohorts (**Figures 1 and 2**). The strongest up-regulated proteins (with an absolute log FC ≥1.19 and FDR ≤−0.50) were fibroblast growth factor (FGF)-21 and -23 (log FC 1.47 and 2.28, respectively), mucin-16 (MUC16, CA-125) (log FC 1.83), B-type natriuretic peptide (BNP) (log FC 1.41), interleukin (IL)-6 (log FC 1.28), IL1 receptor-like 1 (IL1RL1) (soluble suppressor of tumorigenicity 2 [sST2]) (log FC 1.27), fatty acid-binding protein 4 (FABP4) (logFC 1.26), and growth differentiation factor (GDF)-15 (log FC 1.19) (**Figure 2A**). The strongest down-regulated proteins in severely congested patients in the index cohort were Kit ligand (KITLG) (log FC −0.52), epidermal growth factor (EGF) (log FC −0.55), and paraoxonase 3 (PON3) (log FC −0.57). Only PON3 was significant in the validation cohort (**Figure 2B**). After adjusting for age and sex, the majority of these differences remained statistically significant (Supplemental Figures 2 and 3).

PATHWAY OVERREPRESENTATION ANALYSES. After performing differential expression analysis, the up-regulated/down-regulated proteins in severely congested patients were regrouped according to their known biological functions ("pathways") based on previous experimental data. The previous experimental data used for comparison belonged to the GO, KEGG, and Reactome data sets. These databases categorize proteins into pathways based on their shared functions. Pathway analyses of the 51 proteins that were significantly up-regulated in both index and validation cohorts are presented in the **Central Illustration**. In total, 22 biological pathways were identified (Supplemental Table 7). The 4 strongest pathways associated with severe congestion were regulation of lymphocyte activation and proliferation, regulation of inflammatory response, cytokine-viral-receptor interaction, and response to mechanical stress (all $P < 0.001$) (**Central Illustration,** Supplemental Table 7). The overrepresented pathways based on the 107 up-regulated proteins of the index cohort overlapped with the pathways based on the 51 commonly up-regulated proteins in both cohorts (**Central Illustration,** Supplemental Figure 4), and the validation cohort showed identical results. Using the 6 down-regulated proteins in the index cohort, we identified the following down-regulated pathways: platelet activation, glioma, and melanoma (Supplemental Figure 5). The 2 proteins down-regulated in the validation cohort and the 1 protein common in both cohorts were not quantitatively sufficient for further pathway analysis (**Figure 1**). Interestingly, tumor necrosis factor receptor superfamily members (TNFRSFs), especially TNFRSF 1A/1B, 6B, 10B, 11A, 13B, 14, and 19, and IL-6 played a role in several pathways (Supplemental Figure 6, Supplemental Table 7).

OUTCOME ANALYSES. Cox regression analyses for both cohorts are presented in Supplemental Tables 8 and 9. In univariable Cox regression analyses,

higher congestion (top CCS group) was significantly associated with an increased risk of all-cause mortality (HR: 3.14 [95% CI: 2.23-4.43]; $P < 0.001$) and the combined end point (HR: 2.67 [95% CI: 2.03-3.51]; $P < 0.001$). Significance was lost after adjusting for the BIOSTAT-CHF risk model. Similar results were observed in the validation cohort.

DISCUSSION

In this study, we performed a large-scale comparison of the biomarker profiles between severely congested and noncongested patients to provide insights into the pathophysiological pathways involved in congestion. In 2 independent cohorts, we found that severe congestion was associated with up-regulation of FGF-21, FGF-23, CA-125, sST2, GDF-15, FABP4, IL-6, and BNP. The key down-regulated biomarkers were KITLG, EGF, and PON3. Overexpressed pathways in severely congested patients were related to inflammation, endothelial activation, and response to mechanical stress.

PROTEIN BIOMARKERS IN CONGESTION. Up-regulated protein biomarkers. From the differential expression plots, we found that FGF-23 was the strongest up-regulated biomarker. FGF-23 is produced by osteocytes and osteoblasts and plays a major role in renal phosphate excretion, sodium reabsorption, and vitamin D metabolism. Elevated FGF-23 levels in HF patients have been associated with fluid overload, worse NYHA functional class, impaired up-titration of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, and worse outcomes.[9,17] MUC16, also known as CA-125, is an emerging congestion biomarker in patients with HF.[18] Traditionally a marker for ovarian cancer, CA-125 is produced by pleural and pericardial

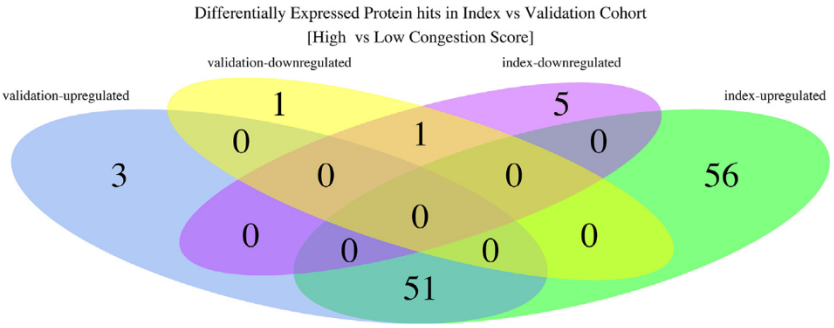
TABLE 1 Continued

	No Congestion (CCS 0) (n = 408)	Severe Congestion (CCS 4 or 5) (n = 142)	*P* Value
Baseline medications			
ACEI/ARB	304 (74.5)	94 (66.2)	0.072
Beta-blockers	363 (89.0)	113 (79.6)	0.007[a]
Diuretics	408 (100)	142 (100)	
Loop diuretics	408 (100)	142 (100)	
Aldosterone antagonists	242 (59.3)	87 (61.3)	0.757
Digoxin	69 (16.9)	43 (30.3)	0.001[a]
Laboratory measurements/biomarkers			
Sodium, mmol/L	140 (138-142)	139 (136-141)	<0.001[a]
Potassium, mmol/L	4.40 (4.10-4.70)	4.10 (3.80-4.42)	<0.001[a]
Hemoglobin, g/dL	13.7 (12.2-14.7)	13.0 (11.3-14.2)	0.001[a]
Total cholesterol, mmol/L	4.53 (3.71-5.31)	3.40 (2.90-4.39)	<0.001[a]
RBCs, 10^{12}/L	4.49 (4.09-4.89)	4.40 (3.98-4.80)	0.184
Platelets, 10^{9}/L	209 (173-254)	198 (159-257)	0.156
Calcium, mmol/L	1.77 (1.49-2.01)	1.72 (1.48-1.96)	0.468
Phosphate, mmol/L	0.83 (0.67-1.03)	0.87 (0.71-1.00)	0.312
Iron, μmol/L	10.0 (6.00-14.0)	6.00 (5.00-10.0)	<0.001[a]
Ferritin, μg/L	121 (51.0-213)	80.0 (44.0-153)	0.008[a]
Transferrin, g/L	2.00 (1.60-2.40)	2.10 (1.70-2.45)	0.062
NT-proBNP, pg/mL	1,564 (673-3,327)	4,041 (2,341-8,866)	<0.001[a]
Troponin T, μg/L	22.4 (13.9-38.7)	39.9 (27.5-62.6)	<0.001[a]
Endothelin-1, pg/mL	4.36 (3.44-5.55)	6.75 (5.77-9.21)	<0.001[a]
Serum creatinine, μmol/L	97.6 (83.5-119)	118 (91.5-151)	<0.001
Urea, mmol/L	9.10 (6.90-15.2)	14.0 (9.00-21.2)	<0.001
Albumin, g/L	34.0 (29.0-39.0)	30.0 (26.0-34.0)	<0.001[a]
Aldosterone, pg/mL	101 (51.0-199)	83.5 (37.8-208)	0.426
Renin, uIU/mL	80.2 (26.2-217)	108 (35.0-363)	0.021[a]
eGFR, CKD-EPI formula, mL/min/1.73 m^2	64.7 (49.3-80.3)	51.4 (34.7-72.7)	<0.001
IL-6, pg/mL	3.40 (1.90-6.00)	9.80 (4.90-19.0)	<0.001[a]

Values are n (%), mean ± SD, or median (IQR), unless otherwise indicated. [a]$P < 0.05$.

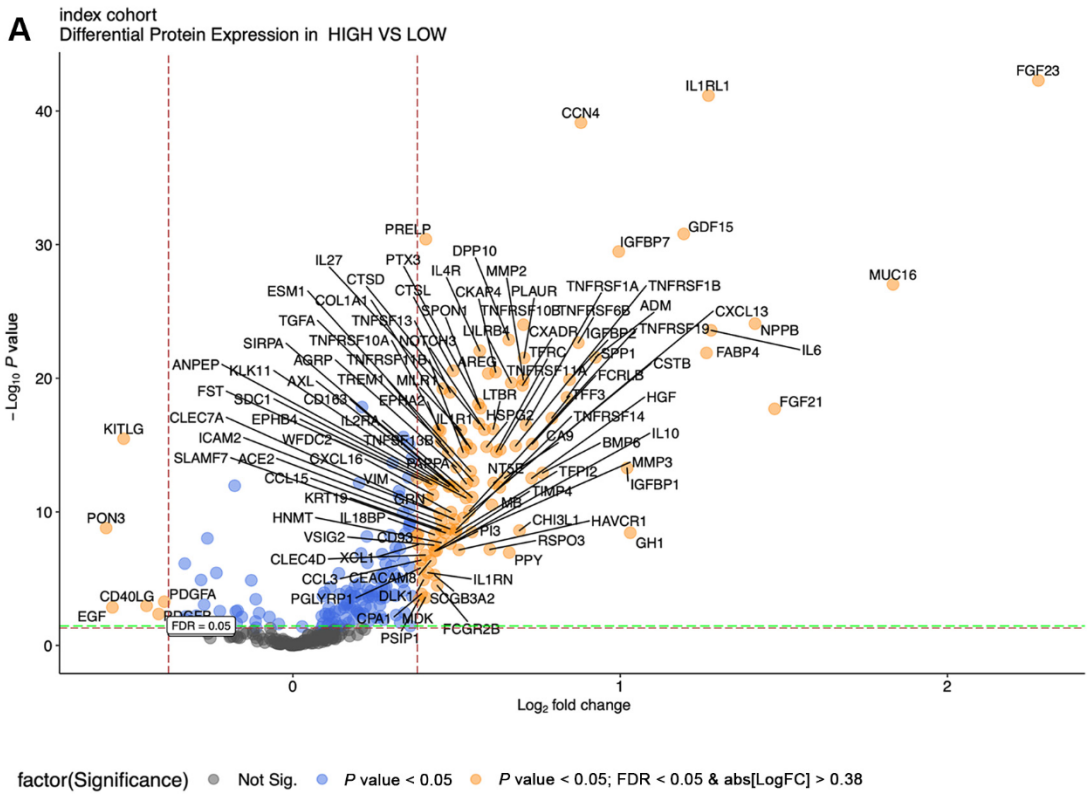
ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; BMI = body mass index; CABG = coronary artery bypass graft surgery; CCS = clinical congestion score; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration; COPD = chronic obstructive pulmonary disease; CRT = cardiac resynchronization therapy; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HF = heart failure; ICD = implantable cardioverter-defibrillator; IL = interleukin; JVP = jugular venous pressure; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro-B-type natriuretic peptide; NYHA = New York Heart Association; PCI = percutaneous coronary intervention; RBC = red blood cell; SBP = systolic blood pressure.

FIGURE 1 Venn Diagram for Differentially Expressed Proteins in the Cohorts



Venn diagram showing the number of up-regulated, down-regulated, and overlapping proteins in highly congested vs noncongested patients in the index and validation cohorts of BIOSTAT-CHF (Biology Study to Tailored Treatment in Chronic Heart Failure).

FIGURE 2 Volcano Plot for Patients With Severe vs No Congestion (Unadjusted)



Volcano plot showing an overview of up-regulated and down-regulated proteins in highly congested vs noncongested patients in the **(A)** index and **(B)** validation cohorts. Biomarkers/proteins above the log2 fold change (FC) of 0.38; $P < 0.05$, and a false discovery rate (FDR)–corrected $P < 0.05$ were considered to be significant. Biomarker/protein FC >0.38 represents up-regulation and FC <−0.38 represents down-regulation. abs = absolute.

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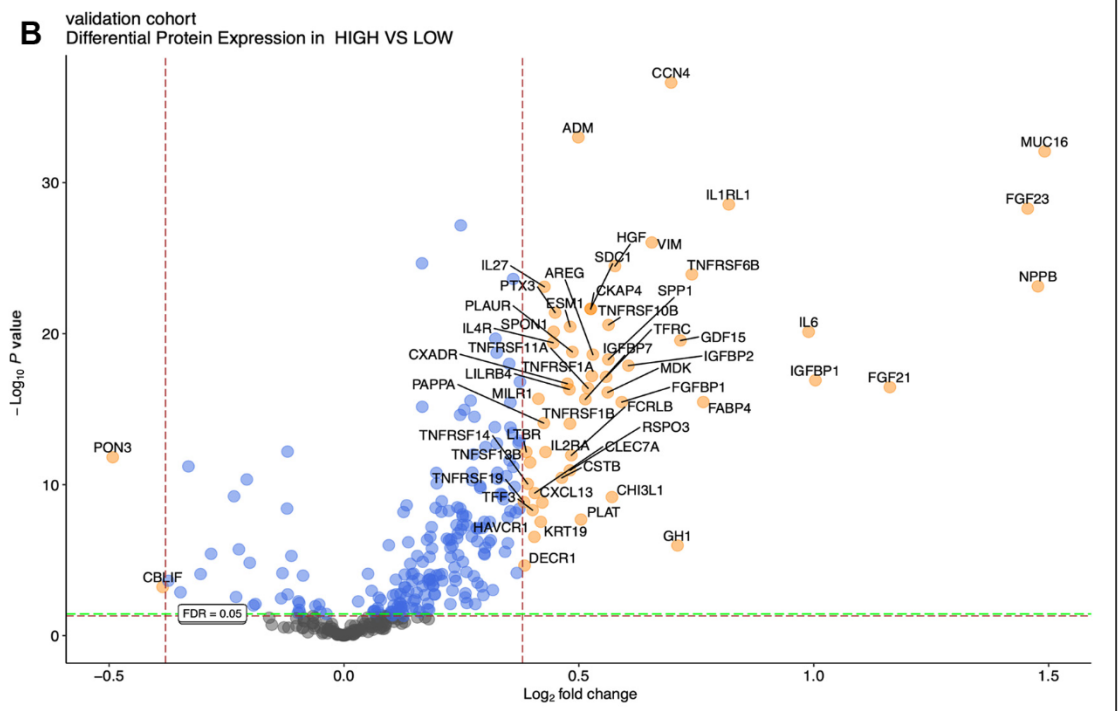
mesothelial cells during hemodynamic and inflammatory stress. CA-125 is a strong predictor of acute HF among outpatients, and biomarker-guided therapy has already been shown to improve renal function parameters and outcomes in acute decompensated HF.[19-22] IL1RL1 (sST2), FGF-21, GDF-15, and FABP4 were among other significant up-regulated biomarkers, previously shown to be involved in inflammation, lipid metabolism, oxidative stress, cardiac remodeling, and worse outcomes.[23-26] Interestingly, IL-6 was the common communicating marker (hub) between several pathways, and has been previously associated with local venous congestion, worsening HF, iron deficiency, and poorer outcomes in HF patients.[27-29] BNP is a well-known marker for cardiac wall stretch during a state of volume and pressure overload.[30] Overall, differential expression profiles showed that the up-regulated biomarkers were predominantly associated with inflammation, oxidative, and hemodynamic stress pathways.

Down-regulated protein biomarkers. We found that KITLG, EGF, and PON3 were the key down-regulated proteins in our cohort. KITLG, otherwise known as stem cell factor or c-Kit ligand, is expressed in mast cells and plays a role in angiogenesis and tissue repair. PON3 is released during a state of oxidative stress and has antiatherosclerotic properties. It promotes high-density lipoprotein formation and inhibits low-density lipoprotein oxidation and monocyte activation. In population-based studies, both KITLG and PON3 were both associated with decreased risk of developing HF, and KITLG was further associated with decreased risk of stroke and myocardial infarction and lower risk of cardiovascular and all-cause mortality.[31-33] EGF helps with vascular

FIGURE 2 Continued



smooth muscle cell contraction and vasoconstriction, and activation of its receptor EGFR is triggered during a state of cardiac remodeling, atherogenesis, and endothelial dysfunction.[34]

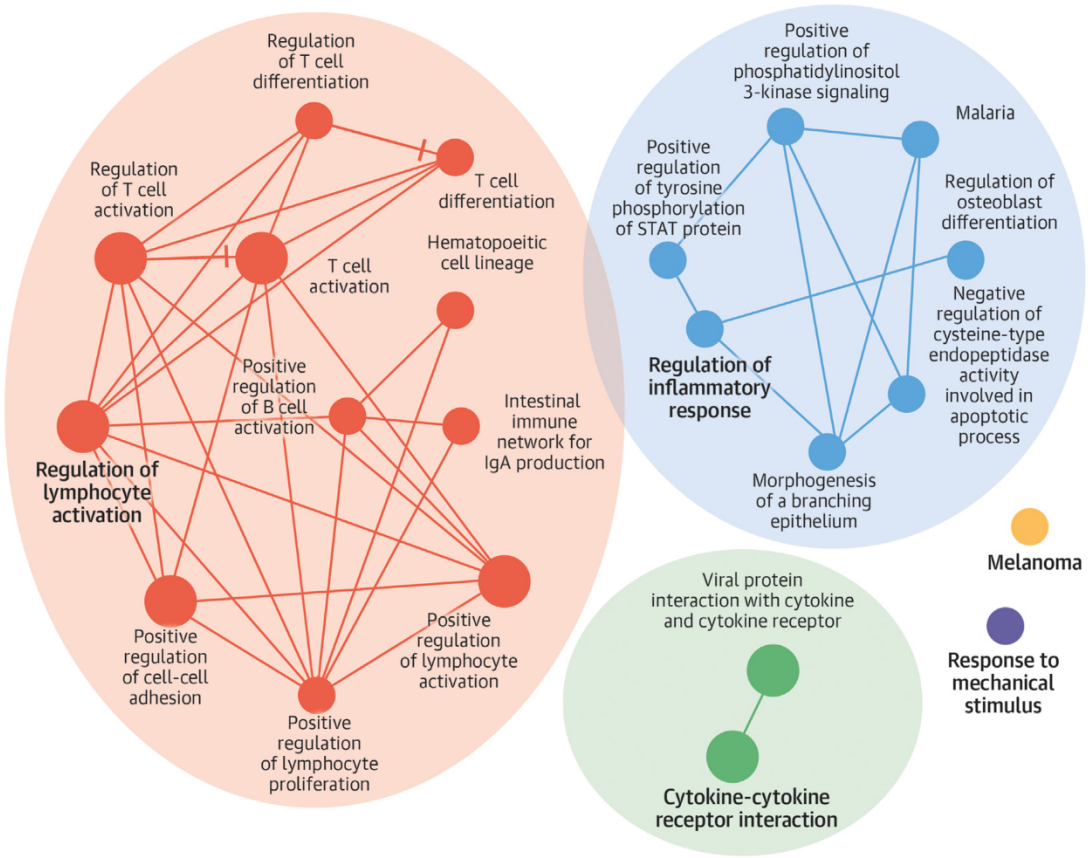
In summary, the identification of these up-regulated and down-regulated proteins in congested HF patients, by means of an unbiased approach, confirms their role as potential congestion markers. The results were consistent in both cohorts and a majority of the markers remained significant even after adjusting for age and sex. The differences in biomarker expression in both cohorts were mainly due to lower NT-proBNP levels and less severe HF in the validation cohort.

INTERPLAY BETWEEN ENDOTHELIAL ACTIVATION, MECHANICAL STRESS, AND INFLAMMATION PATHWAYS. In severely congested patients, we found that pathways related to platelet activation, which is in a subcluster of mechanical stress, were down-regulated, and response to mechanical stimulus pathways were up-regulated. In early stages of HF, impaired cardiac contractility, and activation of sympathetic and neurohormonal response systems, exerts increasing circumferential and sheer stress on peripheral vasculature, thus disrupting a state of homeostasis and switching the endothelial profile from a dormant to an activated state.[1,2] The resulting endothelial dysfunction leads to an increased risk of thrombosis, impaired vasodilation, release of local proinflammatory molecules, transformations in vascular smooth muscle cells, and imbalance of extracellular matrix.[5,6,35,36] Disruption of endothelial barrier function leads to increased permeability (leakiness) of blood vessels, further contributing to local fluid accumulation. Furthermore, we found that pathways related to intestinal immune network for immunoglobulin A production, involved in defense against bacterial translocation, also were up-regulated. In HF, bowel hypoperfusion and edema contribute to gut bacterial overgrowth, epithelial wall damage, and increased bowel permeability. Translocation of bacterial endotoxins further contributes to the immune response and progression of HF.[37,38]

In an experimental study, local peripheral venous congestion, stimulated by inflating a venous pressure arm cuff, was established as a primary source for endothelial and neurohormonal activation and release of proinflammatory cytokines in healthy subjects.[4] This suggests that extensive systemic congestion in HF patients may be a major source of inflammatory and hemodynamic dysfunction, leading to advancement of HF. Because we performed a cross-

JACC: HEART FAILURE VOL. 10, NO. 9, 2022
SEPTEMBER 2022:623–632

CENTRAL ILLUSTRATION Pathways for Proteins Commonly Up-Regulated in Congestion in Index and Validation Cohorts



Pandhi P, et al. J Am Coll Cardiol HF. 2022;10(9):623-632.

Pathway overrepresentation analyses showing the major pathways involved in congestion, based on 51 proteins commonly up-regulated in both cohorts. The larger the size of the node and text, the greater the involvement of the pathway. IgA = immunoglobulin A.

sectional study, it is unknown whether congestion or inflammation was the primary event. However, we do demonstrate a cross-talk between congestion and inflammatory pathways in HF patients at baseline. We found several up-regulated proinflammatory cytokines within the pathways, including IL-6 and TNFRSFs, such as TNFRSF1A, which are known to be strong predictors of worse outcomes in HF patients.[5,27,28] In the early stages of HF, acute inflammation may be cardioprotective by stimulating blood flow and tissue healing, but a sustained low-grade inflammatory state leads to chronic inflammation, causing progressive decompensation.[39] Inflammation itself exerts damaging effects on the heart, leading to cardiomyocyte death, cardiac remodeling, and ventricular dysfunction, further triggering fluid overload. As a result, congestion and inflammation may reciprocally worsen each other.[40]

Because current treatment approaches are mainly focused on reducing fluid overload and sympathetic and neurohormonal response activation, perhaps a multidimensional treatment approach that includes anti-inflammatory therapy may be beneficial. However, trial data on anti-inflammatory treatments have been largely controversial, providing either neutral effects or contributing to worsening of HF condition.

STUDY LIMITATIONS. In this study, we focused on 2 groups of congestion, as defined by the CCS. Orthopnea was not measured in the validation cohort. Congestion was scored at the discretion of the investigating physicians. The group of severely congested patients were predominantly inpatients, which is to be expected, as congestion is often the primary reason for clinical admission. The expressed

biomarker profiles may be a representation of this patient group. We are not able to distinguish between systemic/pulmonary congestion, systolic/diastolic HF, intravascular/tissue congestion, or acute/chronic HF, because the biomarkers are involved in several pathways simultaneously. The results obtained in this study are limited to the 363 proteins/biomarkers measured in HF patients. The human biological system is much more complex than the pathway analyses presented in this study. A more complete representation of pathways would require extensive research on each potential protein/biomarker. Further studies with more proteins from different tissues, preferably based on both experimental and clinical findings, would likely improve our results.

CONCLUSIONS

The present study provides a comprehensive overview of proteins that are up- or down-regulated in patients with severe congestion compared with those without signs of congestion. These proteins may serve as a reliable, low-cost, and objective approach to monitoring the state of congestion in HF patients and may serve as potential therapeutic targets in the near future. The networks of up- and down-regulated proteins suggest that inflammation, endothelial dysfunction, and mechanical stress are associated with severe congestion and provide more insight into the pathophysiology of congestion in HF patients.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Results from this study suggest that congestion is mainly associated with pathways related to inflammation, endothelial activation, and response to mechanical stimulation. The identified highly up-regulated and down-regulated biomarkers may play a diagnostic/prognostic role in the clinical setting and be a potential treatment target for the future. Because current treatment approaches are mainly focused on fluid overload, a more multidimensional treatment approach is called for. It would be interesting to investigate the expression of these protein biomarkers in different HF subgroups, understand how they progress during admissions/outpatient setting, and establish if congestion is the primary event triggering these processes.

TRANSLATIONAL OUTLOOK: Findings on the protein biomarkers, especially the novel down-regulated biomarkers, should be evaluated in an experimental setting within the context of congestion in HF. Future clinical studies or experimental models should focus on whether congestion is the primary source triggering a cascade of biological processes identified in this study.

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APPENDIX For supplemental figures and tables, please see the online version of this paper.