Identifying Pathophysiological Mechanisms in Heart Failure With Reduced Versus Preserved Ejection Fraction

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Abstract
Background. Information on the pathophysiological differences between heart failure (HF) with reduced (HFrEF) versus HF with preserved (HFpEF) ejection fraction is needed
Aims. Establish biological pathways specifically related to HFrEF and HFpEF.
Method. We performed a network analysis to identify unique biomarker correlations in HFrEF and HFpEF using 92 biomarkers from different pathophysiological domains (e.g. inflammation, immune response, metabolic response) in a cohort of 1544 HF patients. Data were independently validated in 804 patients with HF. Networks were enriched with existing knowledge on protein-protein interactions and translated into biological pathways uniquely related to HFrEF, HFmrEF and HFpEF.
Results. In the index cohort (mean age 74 years, 34% female), 718(47%) patients had HFrEF (left ventricular ejection fraction[LVEF]<40%) and 431(27%) patients had HFpEF(LVEF≥50%). 8(12%) correlations were unique for HFrEF and 6(9%) unique to HFpEF. Central proteins in HFrEF were NT-proBNP, growth-differentiation factor-15(GDF15), interleukin-1 receptor type 1(IL1RL1) and activating transcription factor(ATF2), while central proteins in HFpEF were integrin subunit beta-2(ITGB2) and Catenin beta-1(CTNNB1). Biological pathways in HFrEF were related to DNA binding transcription factor activity, cellular protein metabolism and regulation of nitric oxide biosynthesis. Unique pathways in patient with HFpEF were related to cytokine response, extracellular matrix organization and inflammation. Biological pathways of patients with HFmrEF were in between HFrEF and HFpEF.
Conclusion. Network analysis showed that biomarker profiles specific for HFrEF are related to cellular proliferation and metabolism, while biomarker profiles specific for HFpEF are related to inflammation and extracellular matrix reorganization.

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Keywords: HFpEF; HFrEF: network analysis; pathophysiology; biomarkers

Condensed abstract: Pathophysiological differences between patients with heart failure (HF) with reduced (HFrEF) and preserved (HFpEF) are unclear. The present study used network analyses based on 92 biomarkers to identify unique biological mechanisms in patients with HFrEF and HFpEF. Our results suggest that biological processes in HFrEF are associated with DNA binding transcription factor activity, cellular protein metabolism and regulation of nitric oxide, while biological processes in HFpEF are associated with cytokine response, extracellular matrix organization and inflammation. The results of this study stress the pathophysiological heterogeneity between HFrEF and HFpEF and suggest a personalized treatment approach for these patients.

Abbreviations
BIOSTAT-CHF: A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure
GDF15: growth differentiation factor 15
HF: heart failure
HFmrEF: heart failure with a mid-range ejection fraction
HFpEF: heart failure with a preserved ejection fraction
HFrEF: heart failure with a reduced ejection fraction
IL1RL1: Interleukin-1 receptor-like type 1

ITGB2: integrin subunit beta 2
LVEF: left ventricular ejection fraction
NYHA: New York heart association
NT-proBNP: N-terminal B-type natriuretic peptide
PLAUR: plasminogen urokinase receptor
MRA: mineralocorticoid receptor antagonist
STAT1: Signal transducer and activator of transcription 1

Introduction

Heart failure (HF) with a reduced (HFrEF) and preserved (HFpEF) ejection fraction were originally considered as two extremes of the same disease. However, where ACE-inhibitors, angiotensin receptor blockers and mineralocorticoid receptor antagonists are associated with improved clinical outcome in patients with HFrEF (1–3), no such benefit was seen in patients with HFpEF (4–6). It is currently considered that the underlying pathophysiology is different between HFrEF and HFpEF (7–11).

The current paradigm on the underlying pathophysiology of HFpEF suggests that a pro-inflammatory state is responsible for stiffening of the heart muscle and increased filling pressures (7). Indeed, Paulus et al. suggested that the plethora of comorbidities that usually affect patients with HFpEF causes low-level inflammation, which affects the coronary vascular endothelium and reduces nitric oxide bioavailability. His hypothesis suggests that this directly affects the cardiomyocyte and causes cellular hypertrophy as well as cardiac stiffening (7, 12).

Network analysis is a tool to gain novel insights in disease pathways and pathophysiology by studying protein-protein (biomarker-biomarker) correlations (9, 10, 13). By enriching experimentally found protein biomarker networks with knowledge based protein-protein interactions, empirically found correlations can be placed in the context of known pathways (14, 15). We therefore performed a network analysis enriched by knowledge-based interactions to uncover biological mechanisms that are unique for patients with HFrEF and HFpEF.

Methods

Patient population

We studied patients from the BIOSTAT-CHF project, which is described elsewhere (16–20). In brief, BIOSTAT-CHF includes two cohorts of patients with HF included in Scotland and Europe. The aim of the BIOSTAT-CHF study was to characterize biological pathways related to response/no-response to guideline-recommended pharmacological therapy for HF. Therefore, patients had to be sub-optimally treated at inclusion. We used the Scottish cohort of the BIOSTAT-CHF study as our primary study cohort and the European cohort of the BIOSTAT-CHF study as our validation cohort because this was a less selected population. The Scottish cohort consisted of 1738 patients from 6 centers in Scotland, UK. Patients were required to be ≥18 years of age, diagnosed with HF and were previously admitted with HF requiring diuretic treatment. Biomarkers were measured in 1707 of the total of 1738 patients. From these patients, echocardiography was available in 1544 patients. We validated our findings in the European cohort of the BIOSTAT-CHF study, which originally consisted of 2516 patients with HF from 69 centers in 11 European countries. Inclusion criteria for the European cohort include: patients with >18 years of age, having symptoms of new-onset or worsening HF, confirmed either by a LVEF of ≤40% or BNP and/or NT-proBNP plasma levels >400 ng/L or >2,000 ng/L, respectively. Because of this difference in inclusion criteria for patients with LVEF >40%, we excluded all patients with HFrEF and an NT-proBNP level of <2,000 ng/L or patients with HFrEF and no available NT-proBNP levels (**Online Figure 1**). In total, the European cohort consisted of 808 patients with HF with biomarkers available in all patients. All patients needed to be treated with loop diuretics but had not been previously treated with an ACEi/ARBs and/or beta-blocker or they were receiving ≤50% of the target doses of these drugs at the time of inclusion and anticipated initiation or up-titration of ACEi/ARBs and beta-blockers.

Patients in both cohorts had to be sub-optimally treated with ACEi/ARBs and/or beta-blockers, and anticipated initiation or uptitration of ACEi/ARBs and beta-blockers to ESC recommended target doses (21). Furthermore, all patients were enrolled with worsening signs and symptoms of HF as both in-patients or from out-patient clinics (16). To adequately characterize biomarker profiles in patients with HFrEF and HFpEF, we investigated biomarker profiles unique to patients with HFrEF and HFpEF, which showed no overlap with HFmrEF. HFrEF was defined as having an LVEF of <40%, HFmrEF was defined as having an LVEF of 40-49% and HFpEF was defined as having an LVEF of ≥50%.

Clinical and biomarker measurements

Medical history, current use of medication and a physical examination were all recorded at baseline. Standard echocardiography was strongly recommended, but not mandatory for study inclusion. In the combined cohorts, more than 80% of echocardiography were performed within 1 year before inclusion, with more than 70% of echocardiographies performed within 3 months. The timing of echo was similar across HFrEF, and HFpEF in both the Scottish and European cohort.

A large biomarker panel with 92 biomarkers from a wide range of pathophysiological domains were measured in the Scottish and European cohorts. An overview of biomarkers and their pathophysiological function are presented in **Online Table 1.** Assay characteristics are presented in **Online Table 2.** 92 proteins were measured using a high-throughput technique using the Olink Proseek® Multiplex CVD III^{96X96} kit, which measures cardiovascular-related proteins simultaneously in 1µl plasma samples (22). The kit uses a proximity extension assay (PEA) technology, where 92 oligonucleotide-labeled antibody probe pairs are allowed to bind to their respective target present in the sample. PEA is a homogeneous assay that uses pairs of

antibodies equipped with DNA reporter molecules. When binding to their correct targets, they give rise to new DNA amplicons each ID-barcoding their respective antigens.. The amplicons are subsequently quantified using a Fluidigm BioMark™ HD real-time PCR platform. Four internal controls and two external controls (in triplicate) are included in the assay. All information regarding the study population was blind to the laboratory operators.

Statistical analysis

A test for trend was performed to investigate trends in baseline characteristics across HFrEF, HFmrEF and HFpEF. An in-depth description of the methods used for network analysis can be found in the **Online Appendix**. In brief, we performed network analysis using unique pairwise correlations between proteins (biomarkers) within HFrEF, HFmrEF and HFpEF. We retained only those biomarkers which passed the p-value cut-off point following multiple comparisons correction. The p-value cutoff point was based on the number of principal components following principal component analyses (PCA) which determined >95% of the variance among the biomarkers in the separate cohorts (10). A total of 51 PCs, of which the eigenvalues cumulatively explained >95% of the variation observed in the discovery data set were found. To correct for multiple comparison for inter-biomarker correlations, $0.05/([PC\ X\ PC-1]/2)$ was used for the adjusted P cutoff value, where PC is the number of principal components found. This procedure was repeated for the independent European cohort. Here, 50 PCs explained >95% of the variance in the biomarkers. Following, only pairwise correlations were retained that occurred in both the discovery as well as validating cohort. In sensitivity analyses, we tested whether biomarker-biomarker correlations were dependent on NT-proBNP levels by performing separate analyses in patients with NT-proBNP levels above and below 2000 ng/L in the Scottish cohort. Furthermore, in additional sensitivity analyses we tested

whether biomarker-biomarker correlations were similar between patients with HFrEF from the European cohort and patients with HFrEF patients that were excluded based on missing NT-proBNP values or NT-proBNP values below the 2000 ng/L cut off point in our European cohort. Lastly, as an additional sensitivity analysis, we repeated our analyses in patients with HFrEF/HFmrEF/HFpEF included from the out- and inpatient setting alone. To explore whether performing correlation analyses was suitable for our network analyses, we compared the R^2 values to mutual information values according to Steuer et al (23). Due to the difference in N of HFrEF, HFmrEF and HFpEF, correlations retained after a P-value cut-off point had a lower mean R^2 compared to correlations retained in HFmrEF and HFpEF (**Online Figure 1**). To make the correlation networks comparable, an additional cutoff was applied, based on the correlation strength (R^2). To tune the cutoff parameter, the lowest cutoff was chosen that reduces the relation between sample size and R^2, while still retaining a reasonable number of correlations. **Online Figure 2** shows the relation between number of correlations and sample size for six different R2 cutoffs. Based on these observations, a cutoff of $R2 > 0.2$ was chosen. Following, we identified unique correlations between biomarkers for HFrEF and HFpEF, which showed no overlap with HFmrEF and enriched these using knowledge-based protein interactions from a comprehensive list of sources (**Online Appendix**). We then performed pathway overrepresentation analysis to examine overrepresented pathways in HFrEF and HFpEF.

Results

Baseline characteristics

Baseline characteristics are presented in **Table 1.** Overall, patients had a mean age of 73.7 ± 10.7 years and 34.2% were women. Out of a total of 1544 patients, 718 (47%) had HFrEF, 395 (26%) had HFmrEF and 431 (28%) had HFpEF. With increasing LVEF, patients

were older, more often female, had higher rates of diabetes, COPD, hypertension and atrial fibrillation on ECG and were less often on ACEi/ARB and MRA and lower levels of NT-proBNP.

Patients from the European cohort had higher NT-proBNP levels (5122 ng/L vs. 1334 ng/L), other characteristics were generally comparable (**Online Table 3**). Differences between patients according to LVEF strata in the European cohort are presented in **Online Table 4.**

Network analysis

To investigate differences in biomarker profiles between HFrEF and HFpEF, pairwise correlations were extracted that passed a p-value cutoff point corrected for multiple comparisons. We found no high R-squared values with low mutual information values, which suggests that Pearson correlation analyses is suitable (**Online Figure 3**). We studied unique correlation for HFrEF and HFpEF, which showed no overlap with HFmrEF. These pairwise comparisons reflect potential interacting proteins within HFrEF and HFpEF. In total, 65 biomarker correlations passed the p-value cutoff point in HFrEF, HFmrEF and HFpEF in both the Scottish and European cohort (**Figure 1**). Of these, 45 biomarker correlations passed the p-value cut-off point in HFrEF and could be successfully validated in the European cohort. Of these 45 significant correlations, 8 were unique to HFrEF alone (**Figure 1**). Patients with HFpEF showed 40 significant correlations that could be successfully validated, out of the total of 40 correlations, 6 were exclusive to HFpEF (**Figure 1**). There was considerable overlap between HFrEF, HFmrEF and HFpEF with a total of 27 significant correlations that were shared. In sensitivity analyses, biomarker-biomarker correlations were independent of timing of echocardiography and similar in both patients with NT-proBNP levels below and above 2000 ng/L. Furthermore, we found that biomarker-biomarker correlations were similar in patients with HFrEF who were excluded in the

European cohort because of missing NT-proBNP or NT-proBNP values below 2000 ng/L compared to HFrEF patients included. Lastly, sensitivity analyses restricted to in- or outpatients did not affect our results.

Results of the network analyses for HFrEF and HFpEF are presented in **Figure 2 and 3.** The size of the node (hub) is related to the centrality and importance of the hub in the particular network. In other words, biomarkers that form large hubs within a network can be considered biologically more important compared to biomarkers that are smaller hubs. Network analysis showed that main hubs in HFrEF were NT-proBNP, GDF15 and IL1RL1 (**Figure 2A**). In HFpEF, no clear hubs were observed among the unique correlations between the measured biomarkers (**Figure 3A**).

Knowledge based enrichment of network analysis

We enriched the experimentally found networks with protein-protein associated based on various independent databases as described in the **Online Appendix**. By including knowledge-based data-analysis the cyclic AMP-dependent transcription factor ATF2 became an additional hub in HFrEF (**Figure 2B**). When adding knowledge-based interactions to the biomarker networks in HFpEF, integrin subunit beta 2 (ITGB2) and Catenin beta-1, became prominent hubs in HFpEF (**Figure 3B**). In the enriched networks of HFmrEF, we found that plasminogen urokinase receptor (PLAUR), Signal transducer and activator of transcription 1 (STAT1), Transcription factor AP-1 (JUN) and IL-1B were possible hubs (**Online Figure 4**).

Translation into biological pathways

The proteins found in our network analysis which was enriched by existing knowledge on biomarker interactions, were translated into biological pathways that were typically related to HFrEF and HFpEF (**Figure 4***)*. The top 10 overrepresented pathways in HFrEF were

characterized by processes relating to DNA binding transcription factor activity, phosphorylation of peptidyl-serine, cellular protein metabolic processes as well as the regulation in nitric oxide biosynthetic processes. In contrast, the top 10 overrepresented pathways in patient with HFpEF were characterized by inflammatory processes, including cytokine response, extracellular matrix organization as well as response to lipopolysaccharides and inflammation. In HFmrEF, the top 10 upregulated pathways were related to neutrophil degranulation, leucocyte migration and DNA binding transcription factor activity (Online Figure 5).

Discussion

This is the first study using a comprehensive knowledge-based network analysis approach to characterize differences in circulating biomarker signatures among patients with HFrEF, HFmrEF and HFpEF. Overall, there was an important overlap between protein-protein correlations in HFrEF, HFmrEF and HFpEF. This suggests that a large proportion of these protein-protein correlations belong to common pathways related to HF. However, we also found distinct differences, which are summarized in **Figure 5**. Our findings show that pathways specifically up regulated in patients with HFrEF were related to cellular growth and metabolism. Pathways that were specifically up regulated in patients with HFpEF were related to inflammation and extracellular matrix reorganization.

Network analysis of unique biomarker correlations in HFrEF showed that NT-proBNP, GDF15 and IL1RL1 were central hubs. NT-proBNP is associated with cardiac stretch and was previously found to be a specific hub in network analyses in HFrEF in two independent studies (9, 10). GDF15 was previously found to be associated with more adverse outcomes in HFrEF (24, 25). Furthermore, results of our study show that IL1RL1 is a potential hub in patients with HFrEF. In patients with HFmrEF, IL1-B was a hub suggesting that IL1 inhibition in these

patients might be worth investigating. Network analysis in HFpEF showed a more diffuse combination of biomarker correlations with no specific central hubs. This is in line with earlier studies, which suggested that HFpEF might be more heterogenous than HFrEF (26, 27). The majority of biomarkers found in HFpEF were related to inflammation, which is a hallmark of the underlying pathophysiology of HFpEF (7). After adding knowledge-based protein-protein interactions to our experimentally found networks, we observed that ATF2 was an important additional hub in HFrEF. ATF2 is a protein involved in cardiac hypertrophy triggered by TGF-β. A previous experimental study found that suppression of ATF2, attenuated left ventricular hypertrophic response (28). In HFpEF, we observed that ITGB2 and catenin-beta were important hubs. Previous studies show that ITBG2 is involved in chronic inflammatory processes and endothelial dysfunction (29). In addition, an experimental study showed that catenin-β levels were increased in dahl salt-sensitive rats when they developed a HFpEF phenotype (30). This suggests that particularly catenin-β could be a protein of interest in HFpEF. The knowledge-based enrichment of our networks was performed using combined data from various publicly available bioinformatic repositories which together provide a comprehensive data source on all known protein-protein interactions. The combination of these resources reduced overall bias in our enrichment. Yet, without knowledge-based enrichment, HFpEF did not show meaningful hubs. This suggests that the overall pathophysiology of HFpEF is more heterogenous compared to HFrEF.

The last step in our analysis was to perform pathway over-representation analysis of the proteins found in our knowledge enriched networks. Results showed that in HFrEF, biological processes were related to sequence-specific DNA binding, phosphorylation of peptidyl-serine and proliferation of smooth muscle cells. Taken together, these processes are all related to cell

proliferation. Furthermore, biological pathways related to protein kinase B signaling and MAPK cascade were also enriched. Both protein kinase B signaling and MAPK are related to cell proliferation and an increase in metabolism (32,33). In contrast, biological processes in HFpEF related to inflammation, integrin signaling and extracellular matrix organization (33). These data confirm earlier findings regarding HFpEF, but also allows future studies to focus on protein-protein interaction within certain existing pathways such as integrin mediated signaling and extracellular matrix organization (7). Biological pathways upregulated in patients with HFmrEF were in between patients with HFrEF and HFpEF. This is in line with a previous study which suggested that biomarker profiles of patients with HFmrEF are in between patients with HFrEF and HFpEF (34). Our approach might be used to identify HFmrEF patients with a HFrEF-like biomarker profile that could derive more benefit from guideline directed treatment.

This study has several clinical implications. First of all, results of this study provide biological context for the presence of clearly distinct syndromes, which may potentially explain the divergent response to HF therapy. Secondly, processes of cardiac stress response and cell proliferation are enriched in patients with HFrEF, while processes related to inflammation are enriched in HFpEF. Particularly ATF2 could be a potential novel treatment target in HFrEF, while ITGB2 and catenin-beta could be novel treatment targets for HFpEF, which deserves further study.

There are several limitations to this study. First of all, echocardiography was not performed at inclusion. Nevertheless, sensitivity analysis showed that the timing of echo did not influence biomarker levels across HFrEF and HFpEF. Furthermore, we were able to validate our findings in an independent cohort, significantly reducing the potential impact of this limitation. Unfortunately, there were missing values for NT-proBNP in our validation cohort. This might

have introduced a potential bias in our European cohort because these patients had to be excluded. In contrast to our Scottish cohort, our European cohort had patients with both HFrEF and HFpEF with an NT-proBNP value >2000 ng/L. This is a limitation, because it might inflate the type II error. However, this is also a particular strength of this study since protein-protein correlations as well as differences in biomarker levels found for HFrEF and HFpEF in this study are relatively stable throughout the disease severity spectrum. Lastly, patients in BIOSTAT-CHF were sub-optimally treated which might introduce potential bias.

Conclusions

Biological pathways unique to HFrEF are associated with increased metabolism and cellular hypertrophy. A potential novel target for HFrEF is ATF2. Biological pathways unique to HFpEF are related to inflammation, neutrophil degranulation and integrin signaling. Potential novel treatment targets in HFpEF are IGTB2 and catenin-beta. These profound dissimilarities in the underlying biological processes emphasizes the need for distinct drug development programs in HFrEF and HFpEF.

Clinical perspectives

Competency in medical knowledge: Pathophysiological differences between patients with heart failure (HF) with reduced (HFrEF) and preserved (HFpEF) are unclear. Results from this study suggest that patients with HFrEF and HFpEF have distinct differences in key pathophysiological processes. We found that biological processes in HFrEF were associated with DNA binding transcription factor activity, cellular protein metabolism and regulation of nitric oxide, while biological processes in HFpEF are associated with cytokine response, extracellular matrix organization and inflammation. This suggests that a personalized treatment in patients with HFpEF is warranted focusing on inflammation as a central pathophysiological tenet.

Translational outlook: biomarker-based network analyses might help identify novel disease mechanisms and possible novel pathophysiological treatment targets. Although these targets might not directly be translatable to novel drugs, results of network analyses can inform further experimental studies to identify possible causal and mechanistic associations.

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Figure Legends

Figure 1: Venn diagram with unique protein-protein correlations in HFrEF HFmrEF and HFpEF. In total, 6 unique protein-protein correlations were identified in HFpEF, while 8 unique protein-protein correlations were identified in HFrEF.

Figure 2: Network analysis depicting unique protein-protein correlations in HFrEF (A) with knowledge-based interactions (B). Orange nodes are derived from data and blue nodes are knowledge-based correlations. The size of the node corresponds to the betweenness-centrality, which signified the importance of the node in the network. The larger the node, the more important it is to the network. The edges (dotted lines) between the nodes represent the correlation coefficient which is either positive (red) or negative (blue) for empirically derived correlations (orange nodes). In case of knowledge-based nodes (blue), the line signifies a protein-protein interaction.

Figure 3: Network analysis depicting unique protein-protein correlations in HFpEF (A) with knowledge-based interactions (B). Orange nodes are derived from data and blue nodes are knowledge-based correlations. The size of the node corresponds to the betweenness-centrality, which signified the importance of the node in the network. The larger the node, the more important it is to the network. The edges (dotted lines) between the nodes represent the correlation coefficient which is either positive (red) or negative (blue) for empirically derived correlations (orange nodes). In case of knowledge-based nodes (blue), the line signifies a protein-protein interaction.

Figure 4: Pathway over-representation analysis showing biological processes unique to HFrEF (red) and HFpEF (green). The Y-axis signifies the top 10 overrepresented biological processes in HFrEF and HFpEF. + stands for "positive regulation of" in a name of a given GO term. The X-

axis is the -log10 of the p-value; the larger the bar, the more significant is the over presentation of that particular biological process.

Figure 5: Concept figure describing the main findings of this study.

Table 1: Baseline characteristics of the Scottish cohort across LVEF categories.

	HFrEF	HFmrEF	HFpEF	
N	*718*	*395*	*431*	**p-value-$_{trend}$**
Demographics				
Age (years)	72.0 (10.9)	74.9 (10.0)	76.2 (9.9)	**<0.001**
Female sex (%)	188 (26.2%)	137 (34.7%)	187 (43.4%)	**<0.001**
BMI (kg/m2)	28.2 (6.0)	28.9 (5.9)	30.0 (6.8)	**<0.001**
SBP (mmHg)	122.7 (21.3)	127.3 (22.3)	129.9 (23.3)	**<0.001**
DBP (mmHg)	69.8 (12.3)	68.5 (13.1)	68.0 (13.7)	0.006
NYHA class				
Class I	6 (0.8%)	5 (1.3%)	4 (0.9%)	**<0.001**
Class II	337 (46.9%)	160 (40.6%)	136 (31.6%)	
Class III	300 (41.8%)	176 (44.7%)	206 (47.8%)	
Class IV	75 (10.4%)	53 (13.5%)	85 (19.7%)	
LVEF (%)	30.1 (7.3)	43.7 (2.8)	57.3 (6.0)	**<0.001**
Heart rate (bpm)	73.9 (16.5)	72.3 (16.4)	75.0 (15.8)	0.172
Comorbidities n (%)				
Anemia	316 (44.4%)	142 (36.0%)	199 (46.4%)	0.001
Diabetes mellitus	212 (29.6%)	133 (34.0%)	158 (36.9%)	0.009
COPD	110 (15.5%)	61 (15.6%)	110 (25.6%)	**<0.001**
Hypertension	363 (50.8%)	249 (63.2%)	293 (68.0%)	**<0.001**
PVD	144 (20.5%)	88 (23.0%)	116 (27.7%)	0.007
Stroke	117 (16.5%)	84 (21.5%)	84 (19.6%)	0.138
Atrial fibrillation on ECG	199 (27.7%)	136 (34.4%)	162 (37.6%)	<0.001
PCI	132 (18.5%)	80 (20.5%)	74 (17.3%)	0.713
CABG	137 (19.1%)	86 (21.8%)	62 (14.4%)	0.089
Laboratory				
NT-proBNP (ng/L)	1672 (667, 4615)	1209.5 (428.0, 2942.0)	1062 (392, 2820)	**<0.001**
eGFR (mL/min/1.73 m²)	59.8 (43.3, 77.4)	59.7 (42.1, 76.6)	58.4 (42.0, 76.0)	0.310
Urea (mmol/L)	8.6 (6.7, 12.3)	8.6 (6.6, 11.2)	8.6 (6.4, 11.7)	0.289
Hemoglobin (g/dL)	13.6 (4.9)	13.5 (6.6)	13.1 (7.6)	<0.001
Medication n (%)				
ACEi/ARB	538 (74.9%)	274 (69.4%)	268 (62.2%)	**<0.001**
Beta-blocker	570 (79.4%)	293 (74.2%)	257 (59.6%)	**<0.001**
MRA	295 (41.1%)	109 (27.6%)	85 (19.7%)	**<0.001**
Diuretics	712 (99.2%)	391 (99.0%)	425 (98.6%)	0.375

Abbreviations: ACEi, ACE-inhibitor; ARB, angiotensin-receptor blocker; BMI, body mass index; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; LBBB, left bundle branch block; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; MRA, mineralocorticoid receptor antagonist; NYHA, New York heart association; NT-proBNP, N-terminal pro B-type natriuretic peptide; PCI, percutaneous coronary intervention; PVD, peripheral vascular disease; SBP, systolic blood pressure.