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Ferreira, João Pedro; Ouwerkerk, Wouter; Santema, Bernadet T.; van Veldhuisen, Dirk J.; Lang, Chim C.; Ng, Leong L.

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Differences in biomarkers and molecular pathways according to age for patients with HFrEF

João Pedro Ferreira[1]; Wouter Ouwerkerk[2,3]; Bernadet T. Santema[4]; Dirk J. van Veldhuisen[4]; Chim C. Lang[5]; Leong L. Ng[6]; Stefan D. Anker[7]; Kenneth Dickstein[8]; Marco Metra[9]; John G.F. Cleland[10]; Samani J. Nilesh[6]; Gerasimos Filippatos[11]; Joseph-Pierre Aboumsallem[4]; Rudolf A. de Boer[4]; Sylwia Figarska[4]; Iziah E. Sama[4]; Adriaan A. Voors[4]; Faiez Zannad[1]

[1] Université de Lorraine, Inserm, Centre d'Investigations Cliniques- Plurithématique 14-33, and Inserm U1116, CHRU, F-CRIN INI-CRCT (Cardiovascular and Renal Clinical Trialists), Nancy, France.

[2] National Heart Centre Singapore, Hospital Drive, Singapore 169659

[3] Dept of Dermatology, Amsterdam UMC, University of Amsterdam, Amsterdam Infection & Immunity Institute,

[4] Department of Cardiology, University of Groningen, University Medical Centre Groningen, Hanzeplein 1, 9713, GZ, Groningen, the Netherlands.

[5] Division of Molecular and Clinical Medicine, School of Medicine, University of Dundee Ninewells Hospital and Medical School, Dundee, UK.

[6] Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, and NIHR Leicester Biomedical Research Centre, Leicester, UK.

[7] Department of Cardiology (CVK) and Berlin-Brandenburg Center for Regenerative Therapies (BCRT); German Centre for Cardiovascular Research (DZHK) partner site Berlin, Charité - Universitätsmedizin Berlin, Berlin, Germany.

[8] Cardiology Division, Stavanger University Hospital, Postboks 8100, 4068 Stavanger, Norway.

[9] Cardiology, ASST Spedali Civili and Department of Medical and surgical specialties, radiological sciences and public health, University of Brescia, Brescia, Italy.

[10] Robertson Centre for Biostatistics, Institute of Health and Wellbeing, University of Glasgow, Glasgow Royal Infirmary, Glasgow, UK.

[11] National and Kapodistrian University of Athens, School of Medicine, Attikon University Hospital, Athens, Greece.

Contact to:

Dr João Pedro Ferreira

Centre d'Investigation Clinique 1433 module Plurithématique

CHRU Nancy - Hopitaux de Brabois, Institut Lorrain du Coeur et des Vaisseaux Louis Mathieu

4 rue du Morvan, 54500 Vandoeuvre les Nancy

Tel : +33 (0) 3 83 15 73 15

Fax : +33 (0) 3 83 15 73 24

Mail to: j.ferreira@chru-nancy.fr

Abstract

Aims: Elderly patients with heart failure with reduced ejection fraction (HFrEF) have worse prognosis and less often receive guideline-recommended therapies. We aim to better understand the underlying pathophysiological processes associated with aging in HFrEF potentially leading to targeted therapies in this vulnerable population.

Methods and Results: From a panel of 363 cardiovascular biomarkers available in 1,611 patients with HFrEF in the BIOSTAT-CHF index cohort and cross-validated in 823 patients in the BIOSTAT-CHF validation cohort, we tested which biomarkers were dysregulated in patients aged>75yr versus <65yr. Secondly, pathway overrepresentation analyses were performed to identify biological pathways linked to higher plasma concentrations of biomarkers in elderly versus younger patients. After adjustment, multiple test correction (FDR 1%), and cross-validation, 27/363 biomarkers were associated with older age, 22 positively, and 5 negatively. The biomarkers that were positively associated with older age were associated with tumor cell regulation, extra-cellular matrix organization, and inflammatory processes, whereas biomarkers negatively associated with older age were associated with pathways that may point to cell proliferation and tumorigenesis. Among the 27 biomarkers, WFDC2 (WAP Four-Disulfide-Core-Domain-2) – that broadly functions as a protease inhibitor - was associated with older age and had the strongest association with all outcomes. No protein-by-sex interaction was observed.

Conclusions: In elderly HFrEF patients, pathways associated with extra-cellular matrix organization, inflammatory processes, and tumor cell regulation were activated, while pathways associated with tumor proliferation functions were down-regulated. These findings may help in a better understanding of the aging processes in HFrEF and identify potential therapeutic targets.

Key-words: aging; chronological age; biological age; biomarkers; heart failure with reduced ejection fraction.

Translational perspective

Elderly patients with heart failure with reduced ejection fraction (HFrEF) have worse prognosis and less often receive guideline-recommended therapies. Using a large set of circulating proteins, elderly patients had higher concentrations of proteins associated with tumor cell regulation, extra-cellular matrix organization, and inflammatory processes, whereas pathways that may point to cell proliferation and tumorigenesis were down-regulated. WAP Four-Disulfide-Core-Domain-2 was associated with older age and had the strongest association with an increased risk of all outcomes. Understanding the underlying pathophysiological processes associated with aging in HFrEF may potentially lead to targeted therapies in this vulnerable population.

Introduction

The average lifespan of the human population is increasing worldwide. It is estimated that 25% of the world population will be older than 65 years by 2030. Age is the main risk factor for the development of cardiovascular diseases; hence, their prevalence increases dramatically with aging[1]. Heart failure (HF), in particular, is a cardiovascular disease epidemic that affects 1-2% of the adult population in developed countries and rises above 10% among the elderly[2, 3]. Age-related physiological changes, multi-comorbidity, frailty, and polypharmacy, all contribute to a poor prognosis and can alter drug pharmacokinetics (and change the effect of treatments) in elderly HF patients[4]. Moreover, the functional decline is more accelerated in some individuals than in others. In other words, the biological and chronological age are not aligned in all individuals[5]. With the increasing HF prevalence among the elderly, it becomes relevant to better understand the mechanisms underpinning biological aging in this population; as this may lead to novel targeted interventions and therapies that may reduce the burden of the disease.

Circulating biomarkers that reflect the aging process in HF, can provide a useful tool for early detection of high-risk patients regardless of their chronological age. Beyond their prognostic performance, these biomarkers should provide insight into the cellular and molecular mechanisms that underlie aging, disease-specific pathways, and prognosis[6]. Aging affects the levels of circulating proteins that are strongly associated with the prognosis of HF patients[7]. Because single-molecule biomarkers are unlikely to explain the complexity of the aging-related processes, a “multi-omics” approach may provide a more comprehensive “blueprint” of aging[8].

Using two independent cohorts of patients with heart failure with reduced ejection fraction (HFrEF), we sought to investigate the circulating proteomic biomarkers associated

with aging, their prognostic implications and underlying mechanistic pathways using pathway analysis.

Methods

Patient population

BIOSTAT-CHF is a European project that enrolled 2,516 patients with worsening HF on less than guideline-recommended doses of medication from 69 centres in 11 European countries, to investigate the factors predicting the response to attempted up-titration of HF therapies. The design and first results of the study and patients have been published[9].

Index cohort

Briefly, patients were aged ≥18 years with signs and symptoms of worsening HF managed either in an out-patient clinic or hospital ward. The diagnosis of HF was confirmed either by a left ventricular ejection fraction (LVEF) of ≤40% or a BNP and/or NT-proBNP plasma levels >400 pg/mL and/or >2000 pg/mL, respectively. Patients needed to be treated with either oral or intravenous furosemide ≥40 mg/day or equivalent at the time of inclusion. Patients were either treatment naïve with respect to disease-modifying therapies (ACEi/ARBs and beta-blockers) or were receiving <50% of the target doses of at least one of these drugs at the time of inclusion[10]. Patients with an active cancer or with an expected survival of less than six months were excluded from the study. The recruitment period lasted 24 months. The median (pct_{25-75}) follow-up time was 21 (9-26) months. In the present analysis we used the 1,611 patients who had both HFrEF (defined by HF and a LVEF ≤40%) and circulating biomarkers measured. A flow-chart is shown in the **Supplemental Figure 1**.

Validation cohort

Our results were cross-validated (double cross-validation) with the BIOSTAT-CHF validation cohort, that was designed as a multicentre, prospective, observational study consisting of 1,738 patients from six centres in Scotland, UK. Median follow-up was 21 months. Patients from the validation cohort were aged >18 years with a HF diagnosis based on echocardiographic evidence of left ventricular dysfunction or a previous documented admission with HF treated with furosemide ≥20 mg/day or equivalent. They were not previously treated or receiving ≤50% of target doses of ACE inhibitors/ARBs and/or beta-blockers according to the 2008 European Society of Cardiology guidelines. Patients could be enrolled as inpatients or from outpatient clinics[9]. In the present analysis we used the 823 patients who had HFrEF and circulating biomarkers measured (i.e., using the same definitions as in BIOSTAT-CHF index cohort).

Study outcomes

The primary outcome was a composite of heart failure hospitalization and all-cause mortality. The adjudication of HFH was performed by the treating physician. After the trial had ended all medical reports of the mortality events were read and adjudicated based on the medical registries from the case report forms, and the cause of death was ascertained and inserted in the dataset as cardiovascular or non-cardiovascular.

This study was conducted according to good clinical practice guidelines and approved by the relevant Ethics Committee at each institution and by Regulatory Authorities in each country. All patients provided written, informed consent prior to enrolment in the study. This study was conducted in conformity with the principles outlined in the Declaration of Helsinki.

Circulating biomarkers

Four protein biomarker panels with 92 biomarkers each (n =363, due to 5 overlapping

proteins) from a wide range of pathophysiological domains were measured using Olink® technology (CVDII, CVDIII, immune-response, and immuno-oncology panels: https://www.olink.com/). These panels were selected because they contain known human cardiovascular, inflammatory and oncologic markers as well as some exploratory human proteins which may have potential as new markers of cardiovascular disease. The biomarkers were measured (in each panel) using a high-throughput technique using the Olink Proseek® Multiplex 96x96 kit, which measures 92 manually-selected proteins simultaneously in 1µl of plasma. 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. The platform provides Log2 normalized protein expression (NPX) data wherein a high protein value corresponds to a high protein concentration, but not an absolute quantification.

The collection of the clinical, biological and biomarker data presented in this analysis was performed at baseline i.e. in the first study visit.

Statistical and bioinformatics analyses

Population description and comparison of the patients` characteristics by tertiles of age (<65yr; 65-75yr; and >75yr) was performed using parametric or non-parametric tests, as appropriate.

Multinomial logistic regression models were used with the younger age category (<65yr) set as reference and the older age category (>75yr) set as the main comparator to

obtain a comparison of the elderly vs. young. The age models were extensively adjusted for potential confounders including, sex, body mass index (BMI), heart rate, systolic blood pressure (SBP), peripheral edema, ischemic etiology, prior revascularization, atrial fibrillation, diabetes, chronic obstructive pulmonary disease (COPD), anemia, urea, renal function (estimated glomerular filtration rate, eGFR by the CKD-EPI formula), albumin, outpatient vs. inpatient setting, NYHA functional class, prior HF hospitalization, stroke, and peripheral artery disease. A multiple test correction for false discoveries was applied using the Benjamini-Hochberg method, and a false discovery rate was set at 0.01 (i.e., FDR1%)[11]. The results were cross-validated with the validation cohort i.e., the results were tested in BIOSTAT-CHF index cohort and validated in BIOSTAT-CHF validation cohort and vice-versa. For robustness of results we present (in the manuscript main tables) the biomarkers that passed the adjustment for confounders plus multiple test correction plus the bidirectional cross-validation. To assess if the biomarker expression could vary by sex, we performed a sex-by-biomarker interaction in the model but none was present. Cox proportional-hazards models were used to study the association between the biomarkers (previously found to be independently associated with age) and outcomes; the same adjustment, correction and validation principles were applied. Since proteins were measured using NPX (Normalized Protein eXpression) values on a Log2 scale, the hazard ratio for each protein estimates the increase in the hazards of event associated with a doubling in the protein concentration.

To study the underlying mechanistic pathways of the most dysregulated proteins associated with age, we created a general network of human physical protein-protein interactions (PPIs), 'HsapiensPPI', consisting of unique nodes and PPIs based on data from BIND, BIOGRID, DIP, HPRD, INTACT and pdzBase. Context-specific networks were constructed by selecting nodes and interactions that occur only between members from the

protein list being investigated (N0-networks) and/or by selecting nodes that indirectly interact, one-neighbour-away, with members of the list (N1- networks). Physical cohesiveness of context-specific networks was assigned using the Physical Interaction Enrichment (PIE) procedure that corrects for biased enrichment[12].

Protein interaction networks were plotted using Cytoscape version v3.7.2, and pathway overrepresentation analyses were done using the Cytoscape-plugin ClueGO version v2.5.6 and Cluepedia version v1.5.6[13]. Annotations are based on Gene Ontology, KEGG and Reactome pathways as source databases. These three resources were updated on 26-March-2020 before the analyses. Enrichment analyses settings were as follows: Go-term fusion used to minimize term redundancy, and reported only results $p<0.05$. The rest were left at recommended default settings.

Analyses were performed using Stata® (version 16) and R® (version 3.6.2). The level of significance was based on the assumptions above defined and was generally lower than 0.001.

Data Availability Statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Results

Patient characteristics

Compared with younger patients (<65yr), the older ones (>75yr) were more often female, had lower BMI, eGFR, albumin levels, higher SBP, more severe signs and symptoms of HF and more co-morbid conditions. Similar characteristic patterns were observed in both cohorts. **Table 1** & **Supplemental Table 1**.

Biomarkers independently associated with age

After the extensive adjustment, correction and cross-validation described in the methods section. The biomarkers positively associated with older age (>75yr) were: decorin (DCN), interleukin 17d (IL17D), matrix metalloproteinase 12 (MMP12), insulin growth factor binding-protein 2 (IGFBP2), osteoprotegerin (OPG), metalloproteinase inhibitor 4 (TIMP4), R-spondin-3 (RSPO3), chitotriosidase-1 (CHIT1), myoglobin (MB), neurogenic locus notch homolog protein 3 (NOTCH3), tumor necrosis factor ligand superfamily member 13 (TNFSF13), triggering receptor expressed on myeloid cells 1 (TREM1), fc receptor-like protein 6 (FCRL6), pancreatic prohormone (PPY), stem cell factor (SCF), integrin beta-5 (ITGB5), TF protein (TF), alpha-1-microglobulin/bikunin precursor (AMBP), C-X-C motif chemokine 13 (CXCL13), WAP four-disulfide core domain protein 2 (WFDC2), TGF-beta receptor type-2 (TGFR2), and prolargin (PRELP). **Table 2**.

The biomarkers negatively associated with older age were: receptor tyrosine-protein kinase erbB-3 (ERBB3), c-type lectin domain family 4 (CLEC4G), receptor tyrosine-protein kinase erbB-2 (ERBB2), hydroxyacid oxidase 1 (HAOX1), and signalling threshold regulating transmembrane adaptor 1 (SIT1). **Table 2**. BNP was not independently associated with older age.

The complete biomarker lists are presented in the **Supplemental Material (Tables 2 to 4)**.

Biomarkers independently associated with both age and outcomes

The biomarkers positively associated with the study outcomes were: 1) all-cause death or HF hospitalization: WFDC2, PRELP, TREM1, ITGB5, and CXCL13; 2) all-cause death: WFDC2, DCN, TNFSF13, TREM1, RSPO3, PRELP, CXCL13, ITGB5, OPG, and NOTCH3; 3) cardiovascular death: WFDC2, TNFSF13, TREM1, CXCL13, RSPO3, and ITGB5; 4) non-cardiovascular death:

WFDC2, IGFBP2, TREM1, TNFSF13, DCN, PRELP, OPG, TGFR2, and RSPO3. **Table 3**. WFDC2 had the strongest associations with all the outcomes. SCF was negatively associated with all-cause death alone. The circulating proteins CXCL13 and ITGB5 were associated with cardiovascular death but not with non-cardiovascular death. **Table 3**.

Network Analysis

In the enriched networks and corresponding over-represented pathways, the proteins ERBB2, ERBB3, and CLEC4G played a central role and could point towards cancer-related processes. The remaining proteins with positive associations with older age were mostly implicated in pathways related to tumor cell regulation, collagen metabolism/extra-cellular matrix organization and inflammation. **Figure 1A-C**. The main findings are resumed in the **Central Illustration**.

Discussion

The main finding of the present study is that elderly HFrEF patients (compared to younger patients) showed activated pathways that are linked to tumor cell regulation, collagen metabolism/extra-cellular matrix organization and inflammation. Oppositely, pathways related to cancer and tumorigenesis had lower expression in older HFrEF patients. Furthermore, a higher expression of pro-inflammatory and pro-fibrotic markers were associated with poor outcomes, with the exception of SCF, which higher expression could be associated with a lower risk of subsequent death. WFDC2 was both associated with older age and had the strongest association with all outcomes.

Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and ultimately death[14]. Aging affects the levels of circulating proteins, metabolites and other molecules that may underpin extra-cellular (e.g., collagen integrity),

cellular (e.g., clonal cell expansion), epigenetic (e.g., DNA methylation) and genetic alterations (e.g., telomere attrition, DNA mutations)[8]. A deeper understanding of the interconnectivity of the aging processes is essential for the development of better prevention strategies and pharmaceutical targets. Heart failure develops through complex interactions of the cardiovascular aging process with risk factors (i.a. obesity, hypertension, and atherosclerosis), comorbidities (i.a. anemia, chronic kidney disease, diabetes), and disease modifiers (i.a. sex, genes, therapies)[15]. In this regard, our study analyzed 363 circulating proteins in two independent cohorts of HFrEF to provide a comprehensive and integrative approach on the aging process of HFrEF patients. It should be noted that all these patients already have major hallmarks of biological aging, as they have symptomatic HF and other vascular/atherosclerotic aging features (e.g., myocardial infarction, stroke, peripheral artery disease). However, even in the presence of advanced biological aging (and adjusting for major potential confounders), circulating biomarkers could identify patients with different biological signatures. Proteins as ERBB2, ERBB3, and CLEC4G had a negative association with older age; these proteins point towards cancer-related processes and have been implicated in the pathogenesis of multiple cancer types. Of note, HF and cancer are associated with each other, and recently, it has been appreciated that incident cancer is considerably more common in patients with HF than in age and sex-matched subjects without HF[16-18]. Indeed, it appears this association is stronger in relatively younger patients with HF than in the (very) elderly (>75yr). The mechanisms for this finding are complex, but circulating HF associated factors have been identified, and these have been causally linked to tumor growth. In prevalent HF, cancer associated pathways appear to be upregulated and may be sensed by tumor biomarkers[19]. It is thus possible, albeit speculative, that HFrEF patients reaching very old age are those less likely to have had a previous malignancy, which

may explain the lower expression of some (but not all) cancer-associated proteins and also the long-term survival of these patients.

ERBB2, also commonly referred to as HER2 (from human epidermal growth factor receptor 2) or HER2/neu, is a protein that in humans is encoded by the ERBB2 gene. This gene encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. Amplification and/or overexpression of this gene has been reported in numerous adenocarcinomas (including breast, ovarian, gastric, lung, bladder, prostate, uterus, and bone). In recent years the protein has become an important biomarker and target of therapy for approximately 30% of breast cancer patients and these mutations have shown sensitivity to certain tyrosine kinase inhibitors (e.g., neratinib)[20]. The dual role of ERBB2 in tumor growth and in physiological adaptive reactions of the heart positions ERBB2 at the intersection between cancer and chronic HF. ERBB2-targeted inhibitory therapy of cancer may induce ventricular dysfunction, and activation of ERBB2 for HF therapy may increase the risk of malignancy. However, the molecular processes leading to the activation of ERBB2 in cancer and heart are different, suggesting that it might be feasible to design drugs that specifically target either individual signaling pathway, activating ERBB2 signaling in HF without increasing the risk of cancer[21]. The ERBB3 (erbB family receptor tyrosine kinase 3) or HER3, plays a central role as cell surface receptor for neuregulins and is involved in the regulation of myeloid cell differentiation. Amplification of this gene and/or overexpression of its protein have been reported in numerous cancers, including prostate, bladder, and breast tumors[22]. The CLEC4G encodes a glycan-binding receptor and is a member of the C-type lectin family which plays a role in the T-cell immune response, and binds to mannose, fucose and N-acetylgalactosamine units that expressed in a variety of carcinoma cells[23]. HAOX1 and SIT1 are involved in T-cell regulation and other inflammatory

pathways that might contribute to the pathophysiological features of cardiovascular disease and cancer[16].

The biomarkers positively associated with older age point towards processes linked to chronic low-grade inflammation, fibrosis and excessive oxidative stress superimposed on a limited capacity for cardiac regeneration[24]. Additionally, we have found that WFDC2 and TREM1 were independently associated with both older age and major adverse outcomes, including cardiovascular and all-cause mortality. WFDC2 (or epididymal secretory protein E4) is a member of the group of serine protease inhibitors belonging to the WAP family[25]. The WFDC2 gene is expressed in pulmonary epithelial cells preserving the integrity of tight junctions between epithelial cells, inhibits bacterial growth and prevents invasion by commensal bacteria and mucosal inflammation in colonic epithelial cells[26]. WFDC2 is also considered an important biomarker for an early diagnosis of ovarian cancer[27]. Markedly, WFDC2 is amongst the strongest predictors of poor outcome in HF, again underscoring the intimate relation between HF and cancer[28]. TREM1 stimulates neutrophil and monocyte-mediated inflammatory responses, serving as an amplifier of inflammatory responses that are triggered by bacterial and fungal infections and is a crucial mediator of septic shock[29]. The main pathways associated with these proteins – epithelial cell integrity and inflammatory response to infections - may explain why these proteins were associated with all causes of death, including non-cardiovascular. On the other hand, the circulating proteins chemokine CXCL13 and ITGB5 were strongly associated with cardiovascular death but not with non-cardiovascular death. CXCL13 is a chemokine highly expressed by stromal tissue and follicular dendritic cells, where, along with its receptor CXCR5, it is essential for the guidance of B lymphocytes to secondary lymphoid organs. The CXCL13-CXCR5 chemokine axis has been associated with various inflammatory conditions, and is strongly expressed in

human atherosclerotic lesions and increased in plasma of patients with carotid atherosclerosis; suggesting a potential role for the CXCL13-CXCR5 chemokine axis in atherosclerosis[30, 31]. The integrin ITGB5 is a cell-surface receptor that participates in cell adhesion and cell-surface mediated signalling. Its expression has been associated with arrhythmogenic right ventricular cardiomyopathy, coronary artery disease, atrial fibrillation and HF[32]. In this case, the main pathways associated with these proteins – atherosclerosis and cardiac dysfunction - may explain why these proteins were associated with cardiovascular death but not non-cardiovascular death.

The only protein with a negative association with all-cause death (but not retained for the other outcomes) was SCF (or c-KIT ligand). SCF is required for hematopoiesis and plays an essential role in the regulation of cell survival and proliferation. Downregulation of SCF-c-kit decreased engraftment of transplanted bone marrow stem cells in infarcted rat hearts[33]. These findings may help explaining why higher concentrations of this marker are associated with lower all-cause death rates.

Limitations

Several limitations should be acknowledged in this analysis. This is a post-hoc observational analysis, therefore all limitations inherent to such analyses are applied herein, including the inability to infer causality; these findings should be regarded as hypothesis generating. To be enrolled in BIOSTAT-CHF patients could not have a known active malignancy, hence these findings may not reflect “real-world” HF patients who may have higher risk of cancer. Moreover, the expression of cancer-related biomarkers was not consistent for all the studied biomarkers. For example, ERBB family proteins were negatively associated with older age, but WFDC2 was positively associated with older age and also the study outcomes. Having a population of patients with HFrEF and a malignancy would provide further insight

about the cancer/age-related pathways. These biomarkers are expressed in arbitrary Log2 normalized NPX units (as detailed in the methods section), therefore the inference of a ready-to-use clinical biomarker "unit" is not possible at this stage. Despite having measured 363 circulating proteins, we cannot ascertain whether a wider set of circulating proteins would provide additional information including different age associated-mechanisms. Some of the mechanisms and pathways intersect (e.g., inflammation, atherosclerosis and tumorigenesis) which may reflect the complexity of HFrEF in humans and the interplay between many co-morbid conditions. This study lacks specific cancer diagnosis as it was not designed for that purpose, and many of these patients could have undiagnosed or low-grade cancers that contributed to the expression of some of these markers. The event adjudication ascertained death from cardiovascular and non-cardiovascular causes, but the specific mode of death (e.g., cancer) was not captured. Importantly, we do not have an age/sex matched control group who did not have HF - so we do not know whether these differences are specific for HF or apply more generally to aging. The bioinformatic approach used is limited to the expressed circulating proteins and may not reflect the relevant intra-cellular mechanisms of aging in patients with HFrEF. Lastly, HF may be a survivor characteristic where only people who lived long enough can have HF, hence the lower expression of cancer-related pathways may reflect this survivor bias by selecting only the people who did not die of cancer earlier.

Conclusion

Elderly HFrEF patients may express higher levels of proteins associated with extra-cellular matrix organization, inflammatory processes, and tumor cell regulating functions, and may express lower levels of proteins associated with tumor proliferation functions. The role of

WFDC2 in HFrEF should be furtherly explored. These findings may help in a better understanding of the aging processes in HFrEF and identify potential therapeutic targets.

Disclosures

The authors declare not having conflicts of interest with regards to the content of this manuscript.

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Authors` contribution

JPF performed statistical analysis and drafted the manuscript; WO performed statistical analysis; BTS performed statistical analysis; DJvV revised the manuscript and provided critical input; CCL revised the manuscript and provided critical input; LLN revised the manuscript and provided critical input; SDA revised the manuscript and provided critical input; KD revised the manuscript and provided critical input; MM revised the manuscript and provided critical input; JGFC revised the manuscript and provided critical input; SJN revised the manuscript and provided critical input; GF revised the manuscript and provided critical input; JPA performed statistical analysis and revised the manuscript; RAdB revised the manuscript and provided critical input; SF performed statistical analysis and revised the manuscript; IES performed statistical analysis and revised the manuscript; AAV study oversight and critical revision of the manuscript; FZ study oversight and critical revision of the manuscript.

Bibliography

1. Hamczyk, M. R.; del Campo, L.; Andres, V., Aging in the Cardiovascular System: Lessons from Hutchinson-Gilford Progeria Syndrome. *Annu Rev Physiol* **2018,** *80*, 27-48.
2. Bleumink, G. S.; Knetsch, A. M.; Sturkenboom, M. C.; Straus, S. M.; Hofman, A.; Deckers, J. W.; Witteman, J. C.; Stricker, B. H., Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. *Eur Heart J* **2004,** *25* (18), 1614-9.
3. Conrad, N.; Judge, A.; Tran, J.; Mohseni, H.; Hedgecott, D.; Crespillo, A. P.; Allison, M.; Hemingway, H.; Cleland, J. G.; McMurray, J. J. V.; Rahimi, K., Temporal trends and patterns in heart failure incidence: a population-based study of 4 million individuals. *Lancet* **2017**.
4. Lazzarini, V.; Mentz, R. J.; Fiuzat, M.; Metra, M.; O'Connor, C. M., Heart failure in elderly patients: distinctive features and unresolved issues. *Eur J Heart Fail* **2013,** *15* (7), 717-23.
5. Laurent, S.; Boutouyrie, P.; Cunha, P. G.; Lacolley, P.; Nilsson, P. M., Concept of Extremes in Vascular Aging. *Hypertension* **2019,** *74* (2), 218-228.
6. Jylhava, J.; Pedersen, N. L.; Hagg, S., Biological Age Predictors. *EBioMedicine* **2017,** *21*, 29-36.
7. Voors, A. A.; Ouwerkerk, W.; Zannad, F.; van Veldhuisen, D. J.; Samani, N. J.; Ponikowski, P.; Ng, L. L.; Metra, M.; Ter Maaten, J. M.; Lang, C. C.; Hillege, H. L.; van der Harst, P.; Filippatos, G.; Dickstein, K.; Cleland, J. G.; Anker, S. D.; Zwinderman, A. H., Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. *Eur J Heart Fail* **2017**.
8. Hamczyk, M. R.; Nevado, R. M.; Barettino, A.; Fuster, V.; Andres, V., Biological Versus Chronological Aging: JACC Focus Seminar. *J Am Coll Cardiol* **2020,** *75* (8), 919-930.
9. Voors, A. A.; Anker, S. D.; Cleland, J. G.; Dickstein, K.; Filippatos, G.; van der Harst, P.; Hillege, H. L.; Lang, C. C.; Ter Maaten, J. M.; Ng, L.; Ponikowski, P.; Samani, N. J.; van Veldhuisen, D. J.; Zannad, F.; Zwinderman, A. H.; Metra, M., A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail* **2016,** *18* (6), 716-26.
10. McMurray, J. J.; Adamopoulos, S.; Anker, S. D.; Auricchio, A.; Bohm, M.; Dickstein, K.; Falk, V.; Filippatos, G.; Fonseca, C.; Gomez-Sanchez, M. A.; Jaarsma, T.; Kober, L.; Lip, G. Y.; Maggioni, A. P.; Parkhomenko, A.; Pieske, B. M.; Popescu, B. A.; Ronnevik, P. K.; Rutten, F. H.; Schwitter, J.; Seferovic, P.; Stepinska, J.; Trindade, P. T.; Voors, A. A.; Zannad, F.; Zeiher, A.; Bax, J. J.; Baumgartner, H.; Ceconi, C.; Dean, V.; Deaton, C.; Fagard, R.; Funck-Brentano, C.; Hasdai, D.; Hoes, A.; Kirchhof, P.; Knuuti, J.; Kolh, P.; McDonagh, T.; Moulin, C.; Reiner, Z.; Sechtem, U.; Sirnes, P. A.; Tendera, M.; Torbicki, A.; Vahanian, A.; Windecker, S.; Bonet, L. A.; Avraamides, P.; Ben Lamin, H. A.; Brignole, M.; Coca, A.; Cowburn, P.; Dargie, H.; Elliott, P.; Flachskampf, F. A.; Guida, G. F.; Hardman, S.; Iung, B.; Merkely, B.; Mueller, C.; Nanas, J. N.; Nielsen, O. W.; Orn, S.; Parissis, J. T.; Ponikowski, P., ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. In *Eur J Heart Fail*, Netherlands, 2012; Vol. 14, pp 803-69.
11. Green, G. H.; Diggle, P. J., On the operational characteristics of the Benjamini and Hochberg False Discovery Rate procedure. *Stat Appl Genet Mol Biol* **2007,** *6*, Article27.
12. Sama, I. E.; Woolley, R. J.; Nauta, J. F.; Romaine, S. P. R.; Tromp, J.; Ter Maaten, J. M.; van der Meer, P.; Lam, C. S. P.; Samani, N. J.; Ng, L. L.; Metra, M.; Dickstein, K.; Anker, S. D.; Zannad, F.; Lang, C. C.; Cleland, J. G. F.; van Veldhuisen, D. J.; Hillege, H. L.; Voors, A. A., A network analysis to identify pathophysiological pathways distinguishing ischaemic from non-ischaemic heart failure. *Eur J Heart Fail* **2020**.
13. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N. S.; Wang, J. T.; Ramage, D.; Amin, N.; Schwikowski, B.; Ideker, T., Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* **2003,** *13* (11), 2498-504.

14. Lopez-Otin, C.; Blasco, M. A.; Partridge, L.; Serrano, M.; Kroemer, G., The hallmarks of aging. *Cell* **2013,** *153* (6), 1194-217.
15. Triposkiadis, F.; Xanthopoulos, A.; Butler, J., Cardiovascular Aging and Heart Failure: JACC Review Topic of the Week. *J Am Coll Cardiol* **2019,** *74* (6), 804-813.
16. Aboumsallem, J. P.; Moslehi, J.; de Boer, R. A., Reverse Cardio-Oncology: Cancer Development in Patients With Cardiovascular Disease. *J Am Heart Assoc* **2020,** *9* (2), e013754.
17. de Boer, R. A.; Meijers, W. C.; van der Meer, P.; van Veldhuisen, D. J., Cancer and heart disease: associations and relations. *Eur J Heart Fail* **2019,** *21* (12), 1515-1525.
18. Meijers, W. C.; de Boer, R. A., Common risk factors for heart failure and cancer. *Cardiovasc Res* **2019,** *115* (5), 844-853.
19. Meijers, W. C.; Maglione, M.; Bakker, S. J. L.; Oberhuber, R.; Kieneker, L. M.; de Jong, S.; Haubner, B. J.; Nagengast, W. B.; Lyon, A. R.; van der Vegt, B.; van Veldhuisen, D. J.; Westenbrink, B. D.; van der Meer, P.; Sillje, H. H. W.; de Boer, R. A., Heart Failure Stimulates Tumor Growth by Circulating Factors. *Circulation* **2018,** *138* (7), 678-691.
20. Hynes, N. E.; Stern, D. F., The biology of erbB-2/neu/HER-2 and its role in cancer. *Biochim Biophys Acta* **1994,** *1198* (2-3), 165-84.
21. Vermeulen, Z.; Segers, V. F.; De Keulenaer, G. W., ErbB2 signaling at the crossing between heart failure and cancer. *Basic Res Cardiol* **2016,** *111* (6), 60.
22. Braunstein, E. M.; Li, R.; Sobreira, N.; Marosy, B.; Hetrick, K.; Doheny, K.; Gocke, C. D.; Valle, D.; Brodsky, R. A.; Cheng, L., A germline ERBB3 variant is a candidate for predisposition to erythroid MDS/erythroleukemia. In *Leukemia*, England, 2016; Vol. 30, pp 2242-2245.
23. Liu, W.; Tang, L.; Zhang, G.; Wei, H.; Cui, Y.; Guo, L.; Gou, Z.; Chen, X.; Jiang, D.; Zhu, Y.; Kang, G.; He, F., Characterization of a novel C-type lectin-like gene, LSECtin: demonstration of carbohydrate binding and expression in sinusoidal endothelial cells of liver and lymph node. *J Biol Chem* **2004,** *279* (18), 18748-58.
24. Lakatta, E. G., Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part III: cellular and molecular clues to heart and arterial aging. *Circulation* **2003,** *107* (3), 490-7.
25. Chen, Y.; Huang, L.; Wang, S.; Liu, T.; Wu, Y.; Li, J. L.; Li, M., WAP four-disulfide core domain protein 2 promotes metastasis of human ovarian cancer by regulation of metastasis-associated genes. *J Ovarian Res* **2017,** *10* (1), 40.
26. Parikh, K.; Antanaviciute, A.; Fawkner-Corbett, D.; Jagielowicz, M.; Aulicino, A.; Lagerholm, C.; Davis, S.; Kinchen, J.; Chen, H. H.; Alham, N. K.; Ashley, N.; Johnson, E.; Hublitz, P.; Bao, L.; Lukomska, J.; Andev, R. S.; Bjorklund, E.; Kessler, B. M.; Fischer, R.; Goldin, R.; Koohy, H.; Simmons, A., Colonic epithelial cell diversity in health and inflammatory bowel disease. *Nature* **2019,** *567* (7746), 49-55.
27. Zheng, L. E.; Qu, J. Y.; He, F., The diagnosis and pathological value of combined detection of HE4 and CA125 for patients with ovarian cancer. *Open Med (Wars)* **2016,** *11* (1), 125-132.
28. de Boer, R. A.; Cao, Q.; Postmus, D.; Damman, K.; Voors, A. A.; Jaarsma, T.; van Veldhuisen, D. J.; Arnold, W. D.; Hillege, H. L.; Sillje, H. H., The WAP four-disulfide core domain protein HE4: a novel biomarker for heart failure. *JACC Heart Fail* **2013,** *1* (2), 164-9.
29. Bouchon, A.; Dietrich, J.; Colonna, M., Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. *J Immunol* **2000,** *164* (10), 4991-5.
30. van der Vorst, E. P. C.; Daissormont, I.; Aslani, M.; Seijkens, T.; Wijnands, E.; Lutgens, E.; Duchene, J.; Santovito, D.; Doring, Y.; Halvorsen, B.; Aukrust, P.; Weber, C.; Hopken, U. E.; Biessen, E. A. L., Interruption of the CXCL13/CXCR5 Chemokine Axis Enhances Plasma IgM Levels and Attenuates Atherosclerosis Development. *Thromb Haemost* **2020,** *120* (2), 344-347.
31. Smedbakken, L. M.; Halvorsen, B.; Daissormont, I.; Ranheim, T.; Michelsen, A. E.; Skjelland, M.; Sagen, E. L.; Folkersen, L.; Krohg-Sorensen, K.; Russell, D.; Holm, S.; Ueland, T.; Fevang, B.; Hedin, U.; Yndestad, A.; Gullestad, L.; Hansson, G. K.; Biessen, E. A.; Aukrust, P.,

Increased levels of the homeostatic chemokine CXCL13 in human atherosclerosis - Potential role in plaque stabilization. *Atherosclerosis* **2012,** *224* (1), 266-73.
32. Verweij, N.; Eppinga, R. N.; Hagemeijer, Y.; van der Harst, P., Identification of 15 novel risk loci for coronary artery disease and genetic risk of recurrent events, atrial fibrillation and heart failure. *Sci Rep* **2017,** *7* (1), 2761.
33. Shahzad, U.; Li, G.; Zhang, Y.; Li, R. K.; Rao, V.; Yau, T. M., Transmyocardial Revascularization Enhances Bone Marrow Stem Cell Engraftment in Infarcted Hearts Through SCF-C-kit and SDF-1-CXCR4 Signaling Axes. *Stem Cell Rev Rep* **2015,** *11* (2), 332-46.

Table 1. Patients` characteristics by age tertiles (BIOSTAT-CHF)

Patients` characteristics	**<65yr**	**65-75yr**	**>75yr**	**p-value**
N. (Total =2008)	788	673	547	
Age, yr	55.2 ± 7.9	70.2 ± 3.3	80.9 ± 4.0	<0.001
Male	645 (81.9%)	536 (79.6%)	343 (62.7%)	<0.001
BMI, kg/m2	28.7 ± 5.9	28.0 ± 5.0	26.1 ± 4.4	<0.001
Heart rate, bpm	83.5 ± 21.5	80.9 ± 20.7	80.2 ± 19.4	0.007
SBP, mmHg	121.6 ± 21.0	125.1 ± 20.8	126.6 ± 20.7	<0.001
Inpatient	520 (66.0%)	407 (60.5%)	349 (63.8%)	0.091
NYHA III/IV	470 (59.6%)	401 (59.6%)	364 (66.5%)	0.018
Peripheral edema	477 (60.5%)	418 (62.1%)	380 (69.5%)	0.003
Prior HFH (last 12mo)	250 (31.7%)	237 (35.2%)	188 (34.4%)	0.34
Ischemic HF	305 (38.7%)	315 (46.8%)	296 (54.1%)	<0.001
PCI/CABG	226 (28.7%)	245 (36.4%)	207 (37.8%)	<0.001
Atrial fibrillation	248 (31.5%)	345 (51.3%)	274 (50.1%)	<0.001
Diabetes	221 (28.0%)	250 (37.1%)	181 (33.1%)	<0.001
COPD	103 (13.1%)	137 (20.4%)	102 (18.6%)	<0.001
Stroke	51 (6.5%)	68 (10.1%)	59 (10.8%)	0.009
Peripheral arterial disease	67 (8.5%)	76 (11.3%)	56 (10.2%)	0.20
Anemia	101 (12.8%)	168 (25.0%)	156 (28.5%)	<0.001
Urea, mmol/L	14.5 ± 11.3	15.7 ± 10.4	16.3 ± 10.5	0.005
eGFR, ml/min/1.73m2	75.9 ± 21.8	59.7 ± 20.4	52.5 ± 19.5	<0.001
Albumin, g/L	33.9 ± 8.1	32.4 ± 8.5	31.0 ± 8.6	<0.001

NT-pro BNP, pg/mL	1916 (779-4210)	2600 (1106-5639)	3570 (1655-7574)	<0.001

Legend: BMI, body mass index; SBP, systolic blood pressure; HF, heart failure; PCI/CABG, percutaneous coronary intervention/coronary artery bypass grafting; eGFR, estimated glomerular filtration rate using the CKD-EPI formula; HFH, heart failure hospitalization. P-value, p for trend.

Table 2. Biomarkers independently associated with older age and corrected for test multiplicity (FDR1%) in both BIOSTAT-CHF and Dundee-HF (double cross-validation)

Biomarker	Age >75yr vs. <65yr (ref.)	
	Beta coef. (95%CI)	**P-value**
DCN	2.08 (1.62-2.55)	<0.00001
IL17D	1.67 (1.26-2.09)	<0.00001
MMP12	0.60 (0.43-0.77)	<0.00001
IGFBP2	0.62 (0.43-0.81)	<0.00001
OPG	0.70 (0.48-0.91)	<0.00001
TIMP4	0.57 (0.38-0.76)	<0.00001
RSPO3	0.47 (0.31-0.63)	<0.00001
CHIT1	0.26 (0.16-0.37)	<0.00001
MB	0.38 (0.23-0.54)	<0.00001
NOTCH3	0.49 (0.29-0.69)	<0.00001
TNFSF13	0.73 (0.40-1.06)	0.00001
TREM1	0.64 (0.35-0.94)	0.00002
FCRL6	0.42 (0.21-0.62)	0.00007
PPY	0.24 (0.12-0.37)	0.00011
SCF	0.45 (0.22-0.68)	0.00014
ITGB5	0.69 (0.33-1.06)	0.00017
TF	0.68 (0.32-1.04)	0.00024
AMBP	0.94 (0.43-1.44)	0.00026
CXCL13	0.34 (0.15-0.53)	0.00049

WFDC2	0.62 (0.23-1.00)	0.00166
TGFR2	0.52 (0.19-0.85)	0.00182
PRELP	0.72 (0.26-1.18)	0.00213
ERBB3	-1.45 (-1.89 to -1.00)	<0.00001
CLEC4G	-0.91 (-1.22 to -0.59)	<0.00001
ERBB2	-1.04 (-1.40 to -0.67)	<0.00001
HAOX1	-0.20 (-0.29 to -0.11)	0.00002
SIT1	-0.56 (-0.82 to -0.30)	0.00002

The coefficients and respective 95% confidence intervals here shown are those from the BIOSTAT-CHF cohort. These biomarkers were also independently associated with older age at a 1% false discovery rate correction in the Dundee cohort (please see the supplemental material for full results).

Legend: DCN, decorin; IL17D, interleukin 17d; MMP12, matrix metalloproteinase 12; IGFBP2, insulin growth factor binding-protein 2; OPG, osteoprotegerin; TIMP4, metalloproteinase inhibitor 4; RSPO3, R-spondin-3; CHIT1, chitotriosidase-1; MB, myoglobin; NOTCH3, neurogenic locus notch homolog protein 3; TNFSF13, tumor necrosis factor ligand superfamily member 13; TREM1, triggering receptor expressed on myeloid cells 1; FCRL6, fc receptor-like protein 6; PPY, pancreatic prohormone; SCF, stem cell factor; ITGB5, integrin beta-5; TF, TF protein; AMBP, alpha-1-microglobulin/bikunin precursor; CXCL13, C-X-C motif chemokine 13; WFDC2, WAP four-disulfide core domain protein 2; TGFR2, TGF-beta receptor type-2; PRELP, prolargin; ERBB3, receptor tyrosine-protein kinase erbB-3; CLEC4G, c-type lectin domain family 4; ERBB2, receptor tyrosine-protein kinase erbB-2; HAOX1, hydroxyacid oxidase 1; SIT1, signalling threshold regulating transmembrane adaptor 1.

Table 3. Biomarkers independently associated with older age and the study outcomes, fully adjusted and corrected for test multiplicity (FDR1%) both in BIOSTAT-CHF and Dundee-HF

Biomarker	**HR (95%CI)**	**P-value**
All-cause death or HF hospitalization		
WFDC2	2.53 (2.01-3.18)	<0.00001
PRELP	2.15 (1.66-2.79)	<0.00001
TREM1	1.56 (1.33-1.83)	<0.00001
ITGB5	1.55 (1.28-1.87)	0.00001
CXCL13	1.23 (1.12-1.34)	0.00002
All-cause death		
WFDC2	3.34 (2.46-4.52)	<0.00001
DCN	2.54 (1.90-3.38)	<0.00001
TNFSF13	2.28 (1.77-2.95)	<0.00001
TREM1	1.87 (1.52-2.29)	<0.00001
RSPO3	1.24 (1.13-1.36)	<0.00001
PRELP	2.10 (1.51-2.93)	0.00001
CXCL13	1.28 (1.14-1.43)	0.00004
ITGB5	1.66 (1.30-2.12)	0.00005
OPG	1.32 (1.14-1.52)	0.0002
NOTCH3	1.23 (1.07-1.40)	0.0025
SCF	0.76 (0.66-0.88)	0.0003
Cardiovascular death		
WFDC2	2.96 (2.05-4.29)	<0.00001

TNFSF13	2.29 (1.68-3.12)	<0.00001
TREM1	1.79 (1.39-2.31)	0.00001
CXCL13	1.28 (1.12-1.48)	0.0005
RSPO3	1.22 (1.09-1.36)	0.0007
ITGB5	1.65 (1.22-2.22)	0.001
Non-cardiovascular death		
WFDC2	1.79 (1.46-2.20)	<0.00001
IGFBP2	1.61 (1.27-2.05)	0.0001
TREM1	2.03 (1.41-2.91)	0.0001
TNFSF13	2.25 (1.44-3.53)	0.0004
DCN	2.51 (1.50-4.20)	0.0004
PRELP	2.71 (1.52-4.82)	0.0007
OPG	1.53 (1.20-1.96)	0.0008
TGFR2	1.98 (1.30-3.03)	0.0015
RSPO3	1.29 (1.11-1.51)	0.0017

Legend: WFDC2, WAP four-disulfide core domain protein 2; PRELP, prolargin; TREM1, triggering receptor expressed on myeloid cells 1; ITGB5, integrin beta-5; CXCL13, C-X-C motif chemokine 13; DCN, decorin; TNFSF13, tumor necrosis factor ligand superfamily member 13; RSPO3, R-spondin-3; OPG, osteoprotegerin; NOTCH3, neurogenic locus notch homolog protein 3; SCF, stem cell factor.

Figure 1. Overall physical interaction with the age-significant predictors

(A) Protein-pathway representation of the biomarkers positively associated with older age

(B) Protein-pathway representation of the biomarkers negatively associated with older age

(C) “KEGG” molecular interaction, reaction and relation networks of the expressed biomarkers

Legend: Main networks and central-hubs of N1-physical interactions enrichment of age-positive and age-negative protein predictors in patients with heart failure and reduced ejection fraction (n =1,611 in the BIOSTAT-CHF index cohort and n =823 in the BIOSTAT-CHF validation cohort). There are two “main theme” pathways: *constitutive signaling by aberrant PI3K in cancer* and *renal cell carcinoma.*

Annotations are based on *Gene Ontology*, *KEGG* and *Reactome* pathways as source databases. These three resources were updated on 26-March-2020 before the analyses. Software versions ClueGO v2.5.6; CluePedia v1.5.6. P-values were obtained from Go-terms fusion and only those <0.05 are reported.

Central Illustration. Main findings of the study

Legend: ↑, up-regulated; ↓, down-regulated; HFrEF, heart failure with reduced ejection fraction.

These findings can be applied to patients with heart failure and reduced ejection fraction (n =1,611 in the BIOSTAT-CHF index cohort and n =823 in the BIOSTAT-CHF validation cohort).