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► **To cite this version:**

Jasper Tromp, Adriaan A Voors, Abhinav Sharma, João Pedro Ferreira, Wouter Ouwerkerk, et al.. Distinct Pathological Pathways in Patients With Heart Failure and Diabetes. *JACC: Heart Failure*, 2020, 8 (3), pp.234-242. 10.1016/j.jchf.2019.11.005 . hal-03757990

HAL Id: hal-03757990

<https://hal.univ-lorraine.fr/hal-03757990>

Submitted on 22 Aug 2022

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Distinct Pathological Pathways in Patients with Heart Failure and Diabetes

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45 **Acknowledgements:** BIOSTAT-CHF was funded by the European Commission [FP7-242209-
46 BIOSTAT-CHF; EudraCT 2010-020808-29]. Additional funding was provided by Roche
47 diagnostics.

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52 **Disclosures:** AAV received consultancy fees and/or research grants from: Amgen, AstraZeneca,
53 Bayer, Boehringer Ingelheim, Cytokinetics, Myokardia, Novartis, Roche Diagnostics, Servier.
54 D.vV. reports board membership fees/travel expenses from BioControl, Cardiorentis, Johnson &
55 Johnson, Novartis, Vifor, and Zoll Medical. K.D. has received honoraria and/or research support
56 from Medtronic, Boston Scientific St Jude, Biotronik and Sorin (device companies), and Merck,
57 Novartis, Amgen, Boehringer Ingelheim, Astra Zeneca, Pfizer, Bayer, GSK, Roche, Sanofi,
58 Abbott, Otsuka, Leo, Servier, and Bristol Meyers Squibb. C.C.L. received consultancy fees
59 and/or research grants from Amgen, Astra Zeneca, MSD, Novartis, and Servier. S.D.A. reports
60 grants from Vifor and Abbott Vascular, and fees for consultancy from Vifor, Bayer, Boehringer
61 Ingelheim, Brahms, Janssen, Novartis, Servier, Stealth Peptides, and ZS Pharma. M.M. has
62 received consulting honoraria from Amgen, Astra Zeneca, Novartis, Relypsa, and Servier, and
63 speaker's fees from Abbott Vascular and Servier. The remaining authors have nothing to
64 disclose.

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Abbreviations

- AGEs, advanced glycation end products
- CV, cardiovascular
- EGFR, epidermal growth factor receptor
- DM, diabetes mellitus
- FABP, fatty acid binding protein-4
- GDF15, growth differentiation factor 15
- HF, heart failure
- HFpEF, heart failure with preserved ejection fraction
- HFrfEF, heart failure with reduced ejection fraction
- LVEF, left ventricular ejection fraction
- PON3, paraoxonase 3
- PPI, protein-protein interaction
- TNFR, tumor necrosis factor receptor
- TTF3, trefoil factor 3
- CHI3L1, chitinase-3-like protein 1

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Abstract

Background. Diabetes adversely affects clinical outcomes and complicates treatment in patients with heart failure (HF). A clear understanding of the pathophysiological processes associated with type 2 diabetes in HF is lacking.

Methods. We performed network and pathway overrepresentation analyses to identify unique pathological pathways in patients with and without diabetes using 92 biomarkers from different pathophysiological domains measured in plasma samples of 1572 patients with HF (31% diabetes) and reduced ejection fraction (HFrEF; LVEF <40%). We validated our results in an independent cohort of 729 patients (30% diabetes).

Results. We first compared biomarker profiles between HF patients with and without diabetes. Patients with diabetes showed higher levels of galectin-4, GDF-15 and FABP-4 and lower levels of PON-3. We then performed network analyses that revealed that epidermal growth factor receptor (EGFR) and galectin-3 were the most prominent connecting proteins. Translation of these networks to biological pathways revealed that diabetes was associated with inflammatory response and neutrophil degranulation. Diabetes conferred worse outcomes after correction for an established risk model (hazard ratio [HR] 1.20; 95% CI 1.01-1.42).

Conclusion. Concomitant diabetes in patients with HFrEF is associated with distinct pathophysiological pathways related to inflammation, protein phosphorylation and neutrophil degranulation. These data support the evaluation of anti-inflammatory therapeutic approaches, and EGFR in particular, for patients with HF and diabetes.

Funding. This study was funded by the European Commission (FP7-242209-BIOSTAT-CHF; EudraCT 2010-020808-29), Roche diagnostics and AstraZeneca.

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Condensed abstract

A clear understanding of the pathophysiological processes associated with type 2 diabetes in HF is lacking. In 1572 patients with HF (31% diabetes) and reduced ejection fraction (HFrEF; LVEF <40%) and a validation cohort of 729 patients, we measured 92 biomarkers from different pathophysiological domains. Using network and pathways overrepresentation analyses, we identified that EGFR and galectin-3 are possible key proteins involved in the pathophysiology of diabetes in HF and upregulated pathways were associated with inflammatory response and neutrophil degranulation.

188

189 **Introduction.**

190 Diabetes is present in 30 - 40% of patients with heart failure (HF) with reduced ejection fraction
191 (HFrEF)(1–3) and increases the risks of mortality and hospitalization for HF(4–6). Patients with
192 DM have an increased risk of developing HF and patients with HF are at increased risk for
193 developing DM(7, 8) and increases the risks of mortality and hospitalization for HF(4–6).
194 Therefore, we need to better understand potential pathophysiological differences between HF
195 patients with and without diabetes.

196 Network analyses can identify relevant pathophysiologic mechanisms by enriching
197 empirically found biomarkers within the context of disease pathways(9–12). Therefore, we
198 aimed to (1) compare the characteristics of the patients with and without DM, (2) use network
199 analyses to compare biomarker profiles and associated pathways in patients with DM compared
200 to those without DM, which might offer new avenues for potential therapeutic targets.

201

202 **Methods.**

203 *Patient population.*

204 We studied patients from the BIOSTAT-CHF project, which is described elsewhere (13, 14). In
205 brief, BIOSTAT-CHF includes two cohorts of patients with HF. The main aim of BIOSTAT-
206 CHF was to characterize biological pathways related to response/no-response to guideline-
207 recommended pharmacological therapy for heart failure. Our index cohort consisted of 2516
208 patients with HF from 69 centers in 11 European countries. Inclusion criteria for the index cohort
209 include: patients with >18 years of age, having symptoms of new-onset or worsening HF,
210 confirmed either by a LVEF of $\leq 40\%$ or BNP and/or NT-proBNP plasma levels >400 pg/ml or
211 $>2,000$ pg/ml, respectively. All patients were sub-optimally treated with ACEi/ARBs and/or

212 beta-blockers, and anticipated initiation or uptitration of ACEi/ARBs and beta-blockers to ESC
213 recommended target doses. Patients in both the index and validation cohort could be enrolled as
214 in-patients or from out-patient clinics(13). Biomarkers were available in 1572 patients with
215 LVEF <40% of the index cohort (supplementary figure 1)(15). We validated our results in an
216 independent cohort of 1738 patients from 6 centers in Scotland, UK. In total, 729 patients had an
217 LVEF <40% and biomarker data available. Diabetes was defined as having a medical history of
218 diabetes and/or being on anti-diabetic medication.

219

220 *Clinical and biomarker measurements.*

221 Medical history, current use of medication and a physical examination were all recorded at
222 baseline. A large biomarker panel with 92 biomarkers was measured in the index and validation
223 cohort. An overview of biomarkers and their pathophysiological function are presented in
224 *supplementary table 1*. Assay characteristics are presented in *supplementary table 2*. Biomarkers
225 were measured using a high-throughput technique using the Olink Proseek® Multiplex CVD III
226 96x96 kit, which measures 92 manually-selected cardiovascular-related proteins simultaneously
227 in 1µl plasma samples. The kit uses a proximity extension assay (PEA) technology, where 92
228 oligonucleotide-labeled antibody probe pairs are allowed to bind to their respective target present
229 in the sample. PEA is a homogeneous assay that uses pairs of antibodies equipped with DNA
230 reporter molecules. When binding to their correct targets, they give rise to new DNA amplicons
231 each ID-barcoding their respective antigens. The amplicons are subsequently quantified using a
232 Fluidigm BioMark™ HD real-time PCR platform. The platform provides normalized protein
233 expression (NPX) data wherein a high protein value corresponds to a high protein concentration,
234 but not an absolute quantification.

235

236 *Role of funding source*

237 The source of funding did not influence the study design or collection of the data nor the
238 analyses or interpretation of the data or writing of the manuscript.

239

240 *Statistical analysis.*

241 Differences between clinical characteristics of patients with and without diabetes were tested
242 using Student's t-test, the Mann-Whitney-U test or the chi-square-test where appropriate.
243 Differences in expression of biomarkers between patients with and without diabetes was
244 performed using the Linear Models for Microarray data analysis (Limma; software (version
245 3.34.9)(16) with a false discovery rate of 0.05 according to the Benjamini-Hochberg method and
246 a log₂ fold change cutoff of 0.2. In addition, to test independence of associations, we performed
247 multivariable logistic regression analyses to test the independent association of biomarker with
248 diabetes status, correcting for relevant clinical confounders including age, sex, body mass index
249 (BMI), ischemic etiology of HF, history of hypertension and eGFR. Similarly, for both the index
250 and validation cohort, biomarkers that passed the cut-off values for the FDR corrected p-value
251 and log₂ fold change cutoff were then used in subsequent network analyses. To provide
252 biological context to the proteins found, we created a general network of human physical PPI,
253 'HsapiensPPI', consisting of 17,625 unique nodes with 330,157 interactions between them based
254 on data from BIND(17), BIOGRID(18), DIP(19), HPRD(20), INTACT(21) and pdzBase(22)
255 (see supplementary methods). Context-specific networks were constructed by selecting nodes
256 and interactions that occur only between members from the protein list being investigated (N0-
257 networks, colored as orange) and/or by selecting nodes that indirectly interact, one-neighbor-
258 away, with members of the list (N1- networks, colored as blue). Physical cohesiveness of
259 context-specific networks were assigned using the Physical Interaction Enrichment(PIE)

260 procedure that corrects for biased enrichment, in general PPI networks, of proteins that are, e.g.,
261 often studied (23). Analysis of PPIs was performed and plotted using Cytoscape version
262 3.7.0(24), where the node size corresponds to the betweenness centrality(11, 24). A larger node
263 size, the more connected the node is in the network. Pathway overrepresentation analyses was
264 performed using EnrichR, using gene ontology (GO) terms (25, 26). Survival analyses was
265 performed using R version 3.5.1 using the *Survival* and *Survminer* packages(27). Cox regression
266 analyses was used for multivariable survival analyses. Multivariable correction was performed
267 using the BIOSSTAT-CHF risk model, which includes age, blood urea nitrogen (BUN), N-
268 terminal pro-B-type natriuretic peptide (NT-proBNP), hemoglobin (Hb), the use of a beta-
269 blocker at time of inclusion, HF-hospitalization in year before inclusion, peripheral edema,
270 systolic blood pressure, high-density lipoprotein cholesterol and sodium. All tests were
271 performed two-sided and a p-value of smaller than 0.05 was considered statistically significant.

272

273 **Results.**

274 *Baseline characteristics and clinical outcome*

275 Among 1572 patients with HF_{rEF}, 493 (31%) had diabetes. Patients with diabetes were slightly
276 older (69 vs, 67 years, P= 0.002), had a higher BMI (29 vs. 27 kg/m², P <0.001) and had worse
277 signs and symptoms of HF in comparison to patients without diabetes (Table 1). Furthermore,
278 patients with diabetes more often had a history of hypertension (72% vs. 53%, P <0.001)
279 compared to patients without diabetes. Among patients with diabetes, 322 (65%), 183 (37%) and
280 345 (70%) were on oral anti-diabetic medication, insulin or diet controlled respectively.
281 Furthermore, patients with diabetes had worse eGFR (lower), but similar NT-proBNP levels
282 compared to patients without diabetes (Table 1). Patients in the index cohort, were slightly

283 younger and more often in NYHA class III/IV compared to the validation cohort, but had a
284 similar prevalence of comorbidities as well as signs and symptoms (supplementary table 3).

285

286 *Differential protein expression, network and pathway enrichment analyses*

287 In the index cohort, 10 proteins were significantly upregulated and 1 protein was significantly
288 downregulated in patients with diabetes compared to patients without diabetes (Figure 1A & B).

289 In the validation cohort, 13 proteins were significantly upregulated and 3 proteins were
290 significantly downregulated in patients with diabetes compared to patients without diabetes

291 (Figure 1B). In both cohorts, 8 proteins were significantly upregulated. These include Chitinase-
292 3-like protein 1 (CHI3L1), fatty-acid binding protein 4 (FABP4), galectin-4 (GAL4), trefoil

293 factor 3 (TFF3), tumor necrosis factor receptor 1 and 2 (TNFR1 and TNFR2), TNFR super
294 family 14 (TNFRSF14) and growth differentiation factor 15 (GDF15). Paraoxonase 3 (PON3)

295 was downregulated in both cohorts. After additional correction for age, sex, BMI, an ischemic
296 etiology of HF, history of hypertension and eGFR, biomarkers remained differentially expressed

297 in patients with diabetes compared to those without (P for all <0.05). When investigating the
298 association of these biomarkers with diabetic medication (oral vs insulin), levels of TNFR2 (OR:

299 0.76; 95%CI 0.59-0.99) and TNFRSF14 (OR 0.75; 95%CI 0.56-0.98) were slightly lower in
300 patients on oral medication compared to those not on oral medication after correcting for age,

301 sex, BMI, ischemic etiology of HF, history of hypertension, and being on insulins or diet
302 controlled.

303 Results of network analyses are shown in figure 2A. The size of the nodes reflects the
304 number of shortest paths running through the node (edge-betweenness), the larger the node, the

305 higher the number of shortest paths run through the node within the network. In other words, a

306 larger node reflects a greater connectedness within the network. The color of the node relates to
307 either empirically found proteins (orange) or propagated nodes in the N1 network (blue). In the
308 index cohort, epidermal growth factor receptor (EGFR), galectin 3 (GAL3) and granulin (GRN)
309 were important nodes (hubs) in the network of diabetics (Figure 2A). A summary of the proteins
310 measured and their respective functions can be found in supplementary table 1.

311 We then performed functional classification of our N1 networks (Figure 2A) by
312 contrasting the networks against gene ontology terms. The top 10 enriched pathways implicated
313 several key pathways involved in the pathophysiology of HF. Pathways relating to positive
314 regulation of intracellular transduction as well as inflammatory response, neutrophil
315 degranulation and neutrophil mediated immunity were overrepresented (figure 2B).

316

317 *Clinical outcomes*

318 Compared to patients without diabetes, patients with diabetes were more likely to die or get
319 hospitalized for HF within 2 years (hazard ratio [HR] 1.32; 95%CI 1.06-1.66, survival curve as
320 shown in Supplementary figure 2). A similar increase in risk for death and/or heart failure
321 hospitalization was found in the validation cohort (HR:1.63; 95%CI 1.28-2.08, $p < 0.0001$).
322 Among patients who died, patients with DM died equally of CV causes compared to those
323 without DM in both the index (68% vs 68%, $P = 0.987$) and validation cohort (68% vs 65%, $P =$
324 0.77). After correction for the BIOSTAT risk model, patients with diabetes were at higher risk
325 for the primary composite outcome in the index cohort (HR 1.20; 95%CI 1.01-1.42). However,
326 this association was attenuated in the validation cohort (HR 0.92; 95%CI 0.42-1.97). Additional
327 correction for ischemic etiology of HF attenuated the association in the index cohort (HR 1.15;
328 95%CI 0.96-1.36). Of the 10 differentially expression biomarkers, only GDF15 was

329 independently associated with the primary combined outcome after correction for the BIOSTAT-
330 CHF risk model (supplementary table 4). Correcting for GDF15 on top of the clinical risk model,
331 attenuated the association of DM with higher rates of the composite primary outcome ($P = 0.07$).

332 **Discussion.**

333 This is the first study using comprehensive network analyses to distinguish pathophysiological
334 pathways in HF patients with and without diabetes. In two independent cohorts we found that
335 patients with diabetes had higher levels of galectin-4, GDF15 and FABP4 and lower levels of
336 PON3. Furthermore, network analyses showed that EGFR, galectin-3 and granulin are important
337 hubs in HF patients with diabetes. Lastly, we found that specific pathophysiologic processes in
338 diabetes are associated with inflammation and neutrophil degranulation.

339 In a previous study, network analyses was used to compare biomarker profiles between
340 patients with HFrEF and HFpEF (28), where patients with HFpEF had increased inflammation to
341 those with HFrEF. In the present study, patients with diabetes and HFrEF have an upregulation
342 of inflammatory pathways, suggesting that inflammation might also play an important role in
343 patients with HFrEF and diabetes. In the BIOSTAT-CHF study, patients with diabetes and
344 HFrEF were more likely to die or get hospitalized for HF within the first 2 years compared to
345 patients with HFrEF without diabetes. These findings are in line with earlier results from the
346 CHARM-study, which reported similar results (6).

347 A better understanding of the pathophysiology of diabetes in HFrEF is a prerequisite for
348 identifying novel treatment targets and effectively treating diabetes in patients with HFrEF. In
349 the first step of our analyses, we compared biomarker levels between patients with and without
350 diabetes. Our study found that GDF15, galectin-4 and FABP4 were considerably higher in
351 patients with diabetes, while levels of PON3 were lower. Of note, none of the biomarkers

352 identified as hubs within the present study identified patient endotype membership in our
353 previous publication(15), which is also in line with the observation that the prevalence of
354 diabetes did not strongly differ between endotypes. Furthermore, levels of GDF15 are increased
355 in HFrEF and might predict new onset HF in patients with diabetes(10, 31–33). Galectin-4 is
356 involved in inflammation, but no data is available on the role of galectin-4 in patients with
357 concomitant diabetes and HF (34). FABP4 independently predicts left ventricular hypertrophy
358 and dysfunction as well as incident HF in non-diabetic populations (35, 36). PON3 is potentially
359 cardioprotective and attenuates atherogenesis in mice (37). There is evidence that paraoxonases
360 prevent the development of HF and has anti-inflammatory properties, but is often reduced in the
361 presence of atherosclerosis.

362 To provide biological context, we performed network analyses using biomarkers that
363 were differentially expressed between patients with and without diabetes to identify protein-
364 protein interactions in patients with diabetes compared to those without. An earlier study in
365 patients with acute HF from the PROTECT trial, found that among patients with DM, levels of
366 inflammatory markers (TNFR-1a, periostin) and angiogenesis (VEGFR, angiogenin) were
367 significantly increased(9). In addition, network analysis in this study suggested that
368 inflammation and cardiac fibrosis are potentially increased in patients with DM (9). The present
369 studies extends on these previous findings by (1) providing more comprehensive analysis using
370 more biomarkers in a larger number of patients and (2) providing independent validation of our
371 findings. Network analyses revealed that EGFR and galectin-3 were important hubs. Galectin-3
372 is associated with both new onset diabetes as well as severity of HF(38, 39). In HF, galectin-3 is
373 increased and associated with cardiac fibrosis, LV dysfunction and higher mortality(39). EGFR
374 is a transmembrane protein serving as a receptor for epidermal growth factor. Aberrant EGFR

375 signaling is strongly implicated in diabetic nephropathy and HF in experimental studies(41, 42).
376 EGFR inhibition with gefitinib had a protective effect on cardiac remodeling, decreased cardiac,
377 decreased collagen deposition and improved levels of BNP and Troponin-I in a diabetic mouse
378 model(43). In humans, EGFR-inhibitors such as Cetuximab, Panitumumab and Erlotinib are
379 currently used to treat several forms of cancer including breast, colon, lung and pancreatic
380 cancer(44). Taken together, our results suggest that EGFR inhibition in patients with DM and HF
381 might warrant further study.

382 In the following step of our analyses we used pathway enrichment analyses to identify
383 biological pathways upregulated in patients with diabetes. We found that enriched biological
384 pathways in patients with diabetes were associated with inflammatory response and neutrophil
385 degranulation. This is in line with earlier suggestions by Paulus *et al*, where the authors
386 suggested that this excess inflammation might be mediated by advanced glycation end products
387 (AGEs) deposition(47–51). Our results highlight the importance of pathways related to
388 inflammation and EGFR in patients with HFrEF and diabetes, which might warrant future
389 studies into patient specific drug programs involving these pathways.

390

391 **Limitations.**

392 BIOSTAT-CHF is primarily a Caucasian cohort and extrapolation of results to other ethnicities
393 is unclear. Despite rigorous attempts to identify all patients with diabetes, the diagnosis might
394 have been missed in some patients. However, the fact that no non-diabetic patients were on anti-
395 diabetic medications (and even not on diet), makes this unlikely. HbA1c was only available in a
396 limited number of patients. Lastly, no information is available on duration of diabetes, which
397 might have influenced results. Our biomarker panel of 92 proteins is limited. Future studies with

398 a more comprehensive set of proteins or genes would likely provide more pathophysiological
399 differences between diabetic versus non-diabetic HFrEF patients.

400

401 **Conclusion.**

402 In the present study we show that concomitant diabetes in patients with HFrEF is associated with
403 distinct pathophysiological pathways, related to inflammation, protein phosphorylation and
404 neutrophil degranulation. These data support the evaluation of anti-inflammatory therapeutic
405 approaches, and epidermal growth factor receptor in particular, for patients with HFrEF and
406 diabetes.

407

408 **Acknowledgements**

409 BIOSTAT-CHF was funded by the European Commission (FP7-242209-BIOSTAT-CHF;
410 EudraCT 2010-020808-29). Further financial support was provided by Roche diagnostics.

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418 **PERSPECTIVES**

419 **Competency in Medical Knowledge:** Diabetes in patients with HF is associated with unique
420 pathophysiological pathways related to inflammation, protein phosphorylation and neutrophil
421 degranulation, supporting divergent drug development programs for these patients.

422 **Translational Outlook:** Epidermal growth factor receptor and pathways related to inflammation
423 are possible important treatment targets in patients with HF and diabetes, which deserves further
424 study.

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429 **References.**

430 1. Maack C, Lehrke M, Backs J, et al. Heart failure and diabetes: metabolic alterations and
431 therapeutic interventions: a state-of-the-art review from the Translational Research Committee of
432 the Heart Failure Association–European Society of Cardiology. *Eur. Heart J.* 2018;39:4243–
433 4254.

434 2. Seferović PM, Petrie MC, Filippatos GS, et al. Type 2 diabetes mellitus and heart failure: a
435 position statement from the Heart Failure Association of the European Society of Cardiology.
436 *Eur. J. Heart Fail.* 2018;20:853–872.

437 3. Sharma A, Zhao X, Hammill BG, et al. Trends in Noncardiovascular Comorbidities Among
438 Patients Hospitalized for Heart Failure: Insights From the Get With The Guidelines-Heart Failure
439 Registry. *Circ. Hear. Fail.* 2018;11.

440 4. Kristensen SL, Preiss D, Jhund PS, et al. Risk Related to Pre-Diabetes Mellitus and Diabetes
441 Mellitus in Heart Failure with Reduced Ejection Fraction: Insights from Prospective Comparison
442 of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure
443 Trial. *Circ. Hear. Fail.* 2016;9:e002560.

444 5. Domanski M, Krause-Steinrauf H, Deedwania P, et al. The effect of diabetes on outcomes of
445 patients with advanced heart failure in the BEST trial. *J. Am. Coll. Cardiol.* 2003;42:914–922.

446 6. MacDonald MR, Petrie MC, Varyani F, et al. Impact of diabetes on outcomes in patients with
447 low and preserved ejection fraction heart failure: An analysis of the Candesartan in Heart failure:
448 Assessment of Reduction in Mortality and morbidity (CHARM) programme. *Eur. Heart J.*
449 2008;29:1377–1385.

450 7. Lee MMY, McMurray JJ V, Lorenzo-Almorós A, et al. Diabetic cardiomyopathy. *Heart*
451 2019;105:337–345.

452 8. Rosano GM, Vitale C, Seferovic P. Heart Failure in Patients with Diabetes Mellitus. *Card.*
453 *Fail. Rev.* 2017;3:52–55.

454 9. Sharma A, Demissei BG, Tromp J, et al. A network analysis to compare biomarker profiles in
455 patients with and without diabetes mellitus in acute heart failure. *Eur. J. Heart Fail.*
456 2017;19:1310–1320.

- 457 10. Tromp J, Westenbrink BD, Ouwerkerk W, et al. Identifying Pathophysiological Mechanisms
458 in Heart Failure With Reduced Versus Preserved Ejection Fraction. *J. Am. Coll. Cardiol.*
459 2018;72:1081–1090.
- 460 11. Tromp J, Khan MAF, Klip IT, et al. Biomarker profiles in heart failure patients with
461 preserved and reduced ejection fraction. *J. Am. Heart Assoc.* 2017;6:e003989.
- 462 12. Tromp J, Khan MAF, Mentz RJ, et al. Biomarker Profiles of Acute Heart Failure Patients
463 With a Mid-Range Ejection Fraction. *JACC Hear. Fail.* 2017;5:507–517.
- 464 13. Voors AA, Anker SD, Cleland JG, et al. A systems BIOlogy Study to TAIlored Treatment in
465 Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur. J.*
466 *Heart Fail.* 2016;18:716–726.
- 467 14. Ouwerkerk W, Voors AA, Anker SD, et al. Determinants and clinical outcome of uptitration
468 of ACE-inhibitor and beta-blocker in patients with heart failure: a prospective European study.
469 *Eur. Heart J.* 2017;00:1–10.
- 470 15. Tromp J, Ouwerkerk W, Demissei BG, et al. Novel endotypes in heart failure: Effects on
471 guideline-directed medical therapy. *Eur. Heart J.* 2018;39:4269–4276.
- 472 16. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for
473 RNA-sequencing and microarray studies. *Nucleic Acids Res.* 2015;43:e47–e47.
- 474 17. Bader GD, Betel D, Hogue CW V. BIND: the Biomolecular Interaction Network Database.
475 *Nucleic Acids Res.* 2003;31:248–50.
- 476 18. Stark C, Breitkreutz B-J, Reguly T, Boucher L, Breitkreutz A, Tyers M. BioGRID: a general
477 repository for interaction datasets. *Nucleic Acids Res.* 2006;34:D535–D539.
- 478 19. Xenarios I, Rice DW, Salwinski L, Baron MK, Marcotte EM, Eisenberg D. DIP: the database
479 of interacting proteins. *Nucleic Acids Res.* 2000;28:289–91.
- 480 20. Peri S, Navarro JD, Amanchy R, et al. Development of Human Protein Reference Database
481 as an Initial Platform for Approaching Systems Biology in Humans. *Genome Res.*
482 2003;13:2363–2371.
- 483 21. Kerrien S, Aranda B, Breuza L, et al. The IntAct molecular interaction database in 2012.
484 *Nucleic Acids Res.* 2012;40:D841–D846.
- 485 22. Beuming T, Skrabanek L, Niv MY, Mukherjee P, Weinstein H. PDZBase: a protein-protein
486 interaction database for PDZ-domains. *Bioinformatics* 2005;21:827–828.
- 487 23. Sama IE, Huynen MA. Measuring the physical cohesiveness of proteins using physical
488 interaction enrichment. *Bioinformatics* 2010;26:2737–2743.
- 489 24. Shannon P, Markiel A, Ozier O, et al. Cytoscape: A Software Environment for Integrated
490 Models of Biomolecular Interaction Networks. *Genome Res.* 2003;13:2498–2504.
- 491 25. Kuleshov M V., Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set
492 enrichment analysis web server 2016 update. *Nucleic Acids Res.* 2016;44:W90–W97.
- 493 26. Chen EY, Tan CM, Kou Y, et al. Enrichr: interactive and collaborative HTML5 gene list

494 enrichment analysis tool. *BMC Bioinformatics* 2013;14:128.

495 27. Therneau TM. Package “survival”, Version 2.43-3. 2018. Available at: [https://cran.r-](https://cran.r-project.org/package=survival)
496 [project.org/package=survival](https://cran.r-project.org/package=survival). Accessed January 9, 2019.

497 28. Tromp J, Shen L, Jhund PS, et al. Age-Related Characteristics and Outcomes of Patients
498 With Heart Failure With Preserved Ejection Fraction. *J. Am. Coll. Cardiol.* 2019;74:601–612.

499 29. Bao X, Borné Y, Muhammad IF, et al. Growth differentiation factor 15 is positively
500 associated with incidence of diabetes mellitus : the Malmö Diet and Cancer – Cardiovascular
501 Cohort. *Diabetologia* 2018;62:78–86.

502 30. Lee SE, Kang SG, Choi MJ, et al. Growth Differentiation Factor 15 Mediates Systemic
503 Glucose Regulatory Action of T-Helper Type 2 Cytokines. *Diabetes* 2017;66:2774–2788.

504 31. Sharma A, Stevens SR, Lucas J, et al. Utility of Growth Differentiation Factor-15, A Marker
505 of Oxidative Stress and Inflammation, in Chronic Heart Failure: Insights From the HF-ACTION
506 Study. *JACC Hear. Fail.* 2017;5:724–734.

507 32. Dominguez-Rodriguez A, Abreu-Gonzalez P, Avanzas P. Usefulness of Growth
508 Differentiation Factor-15 Levels to Predict Diabetic Cardiomyopathy in Asymptomatic Patients
509 With Type 2 Diabetes Mellitus. *Am. J. Cardiol.* 2014;114:890–894.

510 33. Xu J, Kimball TR, Lorenz JN, et al. GDF15/MIC-1 functions as a protective and
511 antihypertrophic factor released from the myocardium in association with SMAD protein
512 activation. *Circ. Res.* 2006;98:342–350.

513 34. Cao Z-Q, Guo X-L. The role of galectin-4 in physiology and diseases. *Protein Cell*
514 2016;7:314–24.

515 35. Rodríguez-Calvo R, Girona J, Alegret JM, Bosquet A, Ibarretxe D, Masana L. Role of the
516 fatty acid-binding protein 4 in heart failure and cardiovascular disease. *J. Endocrinol.*
517 2017;233:R173–R184.

518 36. Engeli S, Utz W, Haufe S, et al. Fatty acid binding protein 4 predicts left ventricular mass
519 and longitudinal function in overweight and obese women. *Heart* 2013;99:944–948.

520 37. Shih DM, Xia Y-R, Wang X-P, et al. Decreased Obesity and Atherosclerosis in Human
521 Paraoxonase 3 Transgenic Mice. *Circ. Res.* 2007;100:1200–1207.

522 38. Li P, Liu S, Lu M, et al. Hematopoietic-Derived Galectin-3 Causes Cellular and Systemic
523 Insulin Resistance. *Cell* 2016;167:973-984.e12.

524 39. De Boer RA, Van Der Velde AR, Mueller C, et al. Galectin-3: A modifiable risk factor in
525 heart failure. *Cardiovasc. Drugs Ther.* 2014;28:237–246.

526 40. Calvier L, Miana M, Reboul P, et al. Galectin-3 mediates aldosterone-induced vascular
527 fibrosis. *Arterioscler. Thromb. Vasc. Biol.* 2013;33:67–75.

528 41. Li Z, Li Y, Overstreet JM, et al. Inhibition of Epidermal Growth Factor Receptor Activation
529 Is Associated With Improved Diabetic Nephropathy and Insulin Resistance in Type 2 Diabetes.
530 *Diabetes* 2018;67:1847–1857.

531 42. Akhtar S, Yousif MHM, Chandrasekhar B, Benter IF. Activation of EGFR/ERBB2 via
532 Pathways Involving ERK1/2, P38 MAPK, AKT and FOXO Enhances Recovery of Diabetic
533 Hearts from Ischemia-Reperfusion Injury Gallyas F, editor. PLoS One 2012;7:e39066.

534 43. Shah S, Akhtar MS, Hassan MQ, Akhtar M, Paudel YN, Najmi AK. EGFR tyrosine kinase
535 inhibition decreases cardiac remodeling and SERCA2a/NCX1 depletion in streptozotocin
536 induced cardiomyopathy in C57/BL6 mice. Life Sci. 2018;210:29–39.

537 44. Seshacharyulu P, Ponnusamy MP, Haridas D, Jain M, Ganti AK, Batra SK. Targeting the
538 EGFR signaling pathway in cancer therapy. Expert Opin. Ther. Targets 2012;16:15–31.

539 45. Nilsson MB, Sun H, Diao L, et al. Stress hormones promote EGFR inhibitor resistance in
540 NSCLC: Implications for combinations with β -blockers. Sci. Transl. Med. 2017;9:eaao4307.

541 46. Cameron AR, Morrison VL, Levin D, et al. Anti-Inflammatory Effects of Metformin
542 Irrespective of Diabetes Status. Circ. Res. 2016;119:652–65.

543 47. Paulus WJ, Dal Canto E. Distinct Myocardial Targets for Diabetes Therapy in Heart Failure
544 With Preserved or Reduced Ejection Fraction. JACC Hear. Fail. 2018;6:1–7.

545 48. van Heerebeek L, Hamdani N, Handoko ML, et al. Diastolic Stiffness of the Failing Diabetic
546 Heart. Circulation 2008;117:43–51.

547 49. Willemsen S, Hartog JW, van Veldhuisen DJ, et al. The role of advanced glycation end-
548 products and their receptor on outcome in heart failure patients with preserved and reduced
549 ejection fraction. Am. Heart J. 2012;164:742-749.e3.

550 50. Willemsen S, Hartog JW, Hummel YM, et al. Tissue advanced glycation end products are
551 associated with diastolic function and aerobic exercise capacity in diabetic heart failure patients.
552 Eur. J. Heart Fail. 2011;13:76–82.

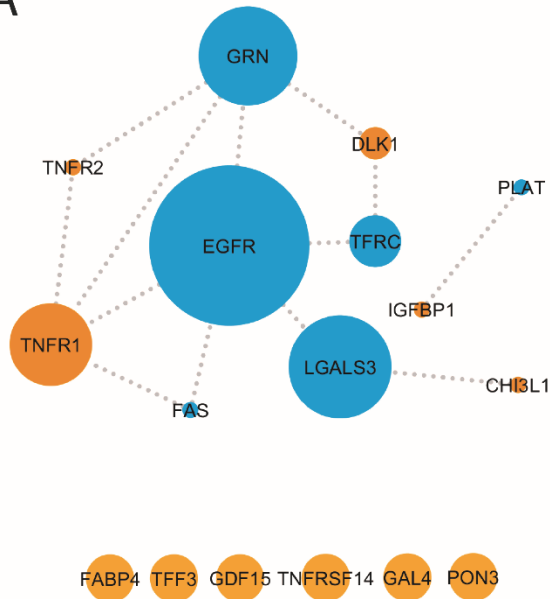
553 51. Willemsen S, Hartog JW, Heiner-Fokkema MR, van Veldhuisen DJ, Voors AA. Advanced
554 glycation end-products, a pathophysiological pathway in the cardiorenal syndrome. Heart Fail.
555 Rev. 2012;17:221–8.

556

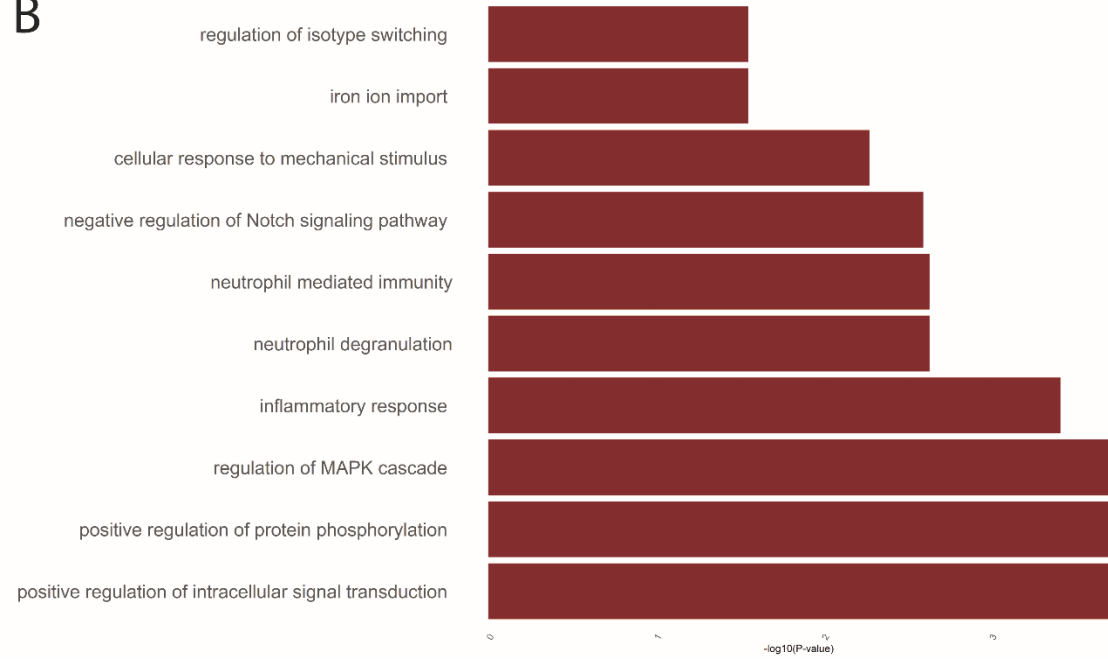
Figure legends

Figure 1: (A) Volcano plot depicting differentially expressed proteins at a log₂ fold change of >0.2 and P-value <0.5 with a false discovery rate (FDR) of <0.05 in the Index cohort. A greater log₂ of the fold change and -log₁₀ of the p-value signifies a greater increase of the mean levels or a more significant difference respectively of each individual biomarker (B) Venn diagram showing the overlap of proteins up and down regulated in patients with HF and diabetes in the index and validation cohort of BIOSTAT-CHF.

A



B



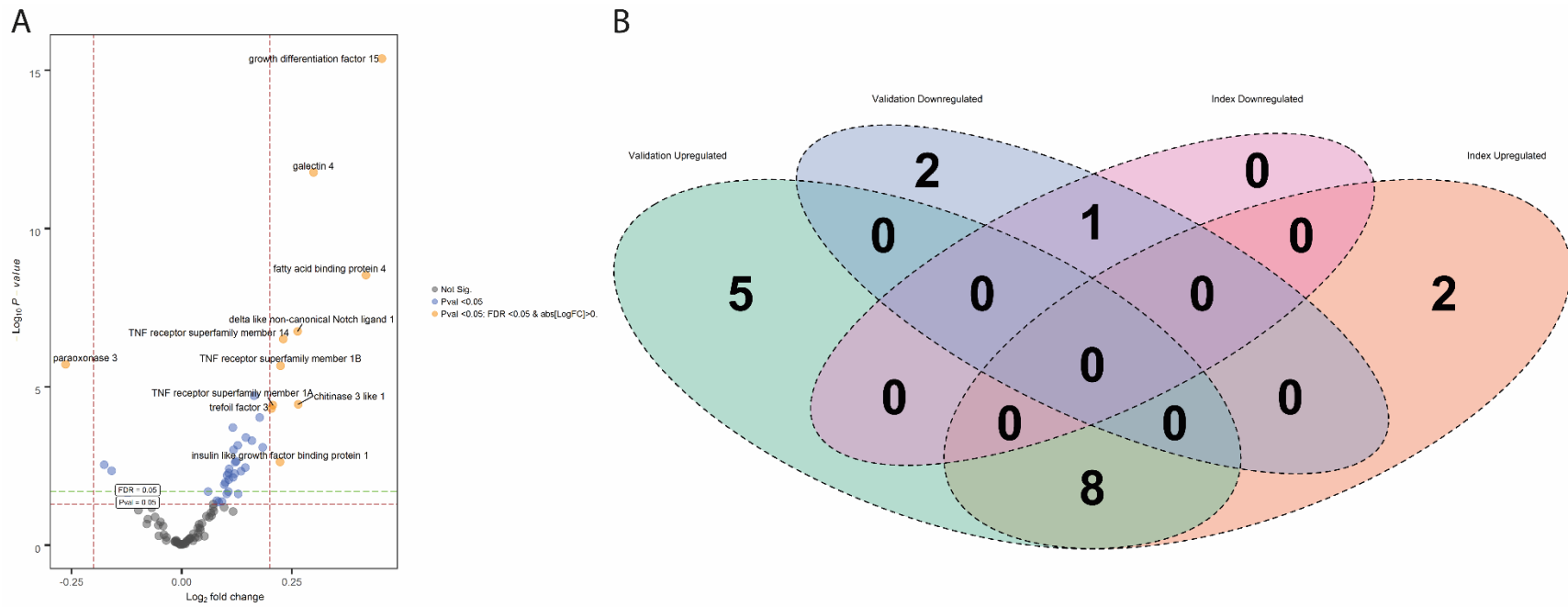


Figure 2/Central illustration: (A) Network of protein biomarkers in patients with diabetes. Orange nodes reflect experimentally found biomarkers. Blue nodes are propagated within the network. The size of the node corresponds to the edge-betweenness (B) overrepresented pathways of protein biomarkers in diabetes.

Table 1: baseline characteristics according to diabetic status.

	No diabetes	Diabetes	p-value
<i>N</i>	1079	493	
<i>Demographics</i>			
<i>Age (years)</i>	66.7 (12.6)	68.8 (10.6)	0.002
<i>Women (%)</i>	269 (24.9%)	103 (20.9%)	0.081
<i>BMI (kg/m2)</i>	26.9 (4.9)	29.1 (5.8)	<0.001
<i>Ischemic etiology (%)</i>	425 (40.2%)	293 (60.5%)	<0.001
<i>LVEF (%)</i>	27.0 (21.0, 32.0)	30.0 (25.0, 35.0)	0.006
<i>NYHA</i>			0.079
<i>I</i>	94 (8.7%)	28 (5.7%)	0.073
<i>II</i>	497 (46.1%)	250 (50.7%)	
<i>III</i>	316 (29.3%)	134 (27.2%)	
<i>IV</i>	32 (3.0%)	22 (4.5%)	
<i>NA</i>	140 (13.0%)	59 (12.0%)	
<i>Systolic BP (mmHg)</i>	122.3 (21.8)	124.2 (19.5)	0.096
<i>Diastolic BP (mmHg)</i>	75.3 (12.9)	74.4 (11.7)	0.17
<i>Heart rate (BPM)</i>	80.6 (19.8)	78.8 (16.7)	0.072

Signs and symptoms			
<i>Elevated JVP</i>	217 (28.4%)	116 (33.0%)	0.14
<i>Hepatomegaly</i>	149 (13.8%)	84 (17.1%)	0.096
<i>Orthopnea</i>	319 (29.6%)	186 (37.7%)	0.001
<i>Edema</i>	453 (51.9%)	257 (61.8%)	0.001
Medical history			
<i>Anemia (%)</i>	289 (28.1%)	203 (42.0%)	<0.001
<i>Atrial fibrillation (%)</i>	447 (41.4%)	219 (44.4%)	0.26
<i>COPD (%)</i>	171 (15.8%)	99 (20.1%)	0.039
<i>Hypertension (%)</i>	569 (52.7%)	357 (72.4%)	<0.001
<i>Peripheral arterial disease (%)</i>	76 (7.0%)	76 (15.4%)	<0.001
<i>Stroke (%)</i>	86 (8.0%)	56 (11.4%)	0.030
Medication			
<i>Oral anti-diabetics (%)</i>	0 (0%)	322 (65.3%)	NA
<i>Insulin use (%)</i>	0 (0%)	183 (37.1%)	NA
<i>Diet controlled (%)</i>	0 (0%)	345 (70%)	NA
<i>Loop diuretics (%)</i>	1075 (99.6%)	490 (99.4%)	0.51
<i>Aldosterone antagonist (%)</i>	599 (55.5%)	273 (55.4%)	0.96
<i>ACEi/ARB (%)</i>	806 (74.7%)	359 (72.8%)	0.4
<i>Beta-blocker (%)</i>	913 (84.6%)	411 (83.4%)	0.438
Laboratory			
<i>Hemoglobin (g/dL)</i>	13.6 (1.8)	13.0 (1.8)	<0.001
<i>Sodium (mmol/L)</i>	140.0 (137.0, 142.0)	139.8 (137.0, 142.0)	0.21
<i>Potassium (mmol/L)</i>	4.3 (3.9, 4.6)	4.2 (3.9, 4.6)	0.91
<i>HbA1c (%)</i>	5.9 (5.5, 6.3)	7.2 (6.5, 8.2)	<0.001
<i>NT-proBNP (ng/L)</i>	4440.0 (2360.0, 8330.0)	3993.0 (2136.0, 8648.0)	0.58
<i>Troponin I (µg/L)</i>	0.0 (0.0, 0.1)	0.1 (0.0, 0.2)	0.001
<i>Glucose (mmol/L)</i>	5.8 (5.2, 6.6)	7.8 (6.3, 10.1)	<0.001
<i>eGFR (mL/min per 1.73 m2)</i>	63.8 (48.0, 80.5)	55.6 (42.7, 74.4)	<0.001

Abbreviations: eGFR, estimated glomerular filtration rate; NT-proBNP, N terminal pro-type natriuretic peptide;

Table 2: summary of novel findings

	Finding
<i>Biomarker levels</i>	<ul style="list-style-type: none"> ↑ galectin-4, GDF-15 and FABP-4 ↓ PON-3
<i>Network analyses</i>	Epidermal growth factor receptor (EGFR) and galectin-3 identified central hubs in the network, signifying possible biological importance
<i>Pathways</i>	<ul style="list-style-type: none"> ↑ Positive regulation of intracellular transduction ↑ Neutrophil degranulation ↑ Neutrophil mediated immunity ↑ Inflammatory response