Biomarker profiles of Acute Heart Failure Patients with a Mid-Range Ejection Fraction

Short title: Biomarkers in acute heart failure with a mid-range ejection fraction.

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Abbreviations:

AHF: acute heart failure.

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.

LVEF: left ventricular ejection fraction.

Abstract.

Objectives: We used biomarker profiles to characterize differences between patients with acute heart failure with mid-range ejection fraction (HFmrEF) and compare them to patients with a reduced (HFrEF) and preserved (HFpEF) ejection fraction.

Background: Limited data is available on biomarker profiles in acute HFmrEF.

Methods: A panel of 37 biomarkers from different pathophysiological domains (e.g., myocardial stretch, inflammation, angiogenesis, oxidative stress, hematopoiesis) were measured at admission and after 24h in 843 AHF patients from the PROTECT trial. HFpEF was defined as LVEF ≥50%(n=108), HFrEF as LVEF <40%(n=607) and HFmrEF as LVEF 40-49%(n=128).

Results: Hemoglobin and BNP levels (300 pg/mL (HFpEF); 397 pg/mL (HFmrEF) 521 pg/mL (HFrEF, p_{trend} <0.001) showed an upward trend with decreasing LVEF. Network analysis showed that in HFrEF interactions between biomarkers were mostly related to cardiac stretch, whereas in HFpEF, biomarker interactions were mostly related to inflammation. In HFmrEF biomarker interactions were both related to inflammation and cardiac stretch. In HFpEF and HFmrEF (but not in HFrEF), remodeling markers at admission and changes in levels of inflammatory markers across the first 24 hours were predictive for all-cause mortality and rehospitalization at 60 days ($P_{interaction}$ <0.05).

Conclusions: Biomarker profiles in patients with acute HFrEF were mainly related to cardiac stretch and in HFpEF related to inflammation. Patients with HFmrEF showed an intermediate biomarker profile with biomarker interactions between both cardiac stretch and inflammation markers.

Keywords: acute heart failure; HFpEF; HFrEF; Biomarkers

Introduction.

Heart failure with a midrange ejection fraction (HFmrEF) has recently been recognized as a new entity within the heart failure (HF) syndrome(1, 2). There is limited understanding of the differences in pathophysiological mechanisms behind HFmrEF, and how these relate to HF with a reduced (HFrEF) and with a preserved (HFpEF) ejection fraction. Previous attempts to understand potential differences in HFrEF and HFpEF have used biomarker-based approaches (3–7). In these conventional biomarker-based studies, baseline biomarker levels and the prognostic value of different biomarkers have been observed between HFrEF and HFpEF (5, 6). However, these approaches were restricted to a limited number of biomarkers measured at a single time point using conventional statistical methods with limited power to uncover underlying pathophysiological differences. Additionally, biomarker profiles of HFmrEF have not been investigated (8–10).

Recently, novel approaches have been useful in increasing the understanding of the pathophysiology of chronic HF by uncovering biomarker associations, previously overlooked by conventional methods (10, 11). In the current study, we aimed to characterize biomarker profiles of patients with HFmrEF and compared these to biomarker profiles of HFrEF and HFpEF (1).

Methods.

Study design and population.

This study was performed in a subcohort of the Patients Hospitalized with acute heart failure and Volume Overload to Assess Treatment Effect on Congestion and Renal FuncTion (PROTECT) trial. The results and methodology of PROTECT have been published previously (12–14). In short, the PROTECT trial was a multicenter, randomized, double blinded, placebo-controlled trial assessing the effect of the Selective A1 Adenosine Receptor Antagonist Rolofylline in 2033 patients

with a history of HF, who were admitted with AHF and mild-moderate renal dysfunction. Patients eligible for inclusion had NT-proBNP levels of >2000 pg/mL with dyspnea at rest or at mild exertion. Patients with severe renal dysfunction or potassium levels below 3.1 mmol/L were excluded (12). Overall results of this trial were neutral (14). Biomarker measurements were performed in 1266 patients. This study assessed a subcohort of 843 patients with available measurements of left ventricular ejection fraction (LVEF) and biomarkers at admission, which were similar in characteristics to the original study population (*supplementary table 1*). Subsequent biomarker samples after 24h were available in 790 patients.

Study measurements and laboratory tests.

Blood sampling was performed at admission before the administration of the study drug and after 24h. Echocardiographic assessment of LVEF was performed at admission or within 6 months prior to admission. A total of 435 (52%) of the echocardiograms were performed at or around admission. HFpEF was defined as having an LVEF ≥50%, while HFrEF was defined as an LVEF <40%. Patients with a LVEF between 40-49% were considered to have HFmrEF (HF with mid-range ejection fraction) (1). A panel of 27 novel and established biomarkers were measured by Alere Inc., San Diego, CA, USA in all available samples. *Table 1* summarizes the biomarkers according to pathophysiological domain. A literature summary for each biomarker was previously performed(11). The classification of biomarkers is based on current literature, however the pathophysiological mechanism behind each biomarker should be judged for each biomarker individually. Galectin-3, Myeloperoxidase (MPO) and Neutrophil gelatinase-associated lipocalin (NGAL) were measured using sandwich enzyme-linked immunosorbent assays (ELISA) on a microtiter plate; Angiogenin and C-reactive protein (CRP) were measured using competitive ELISAs on a Luminex® platform; D-dimer, endothelial cell-selective adhesion molecule (ESAM), growth differentiation factor 15 (GDF-15), lymphotoxin beta receptor (LTBR), Mesothelin, Neuropilin, N-terminal pro C-type natriuretic peptide (NTpro-CNP), Osteopontin, procalcitonin

(PCT), Pentraxin-3, Periostin, PIGR, pro-adrenomedullin (proADM), Prosaposin B (PSAP-B), RAGE, soluble ST2, Syndecan-1, tumor necrosis factor alpha receptor 1 (TNF-R1a), TROY, vascular endothelial growth receptor 1(VEGFR1) and WAP Four-Disulphide Core Domain Protein HE4 (WAP4C) were measured using sandwich ELISAs on a Luminex® platform. A panel of four biomarkers – Endothelin-1 (ET-1), Interleukin-6 (IL-6), Kidney Injury Molecule 1 (KIM-1) and cardiac specific Troponin I (cTnI) was measured in frozen plasma samples collected at baseline using high sensitive single molecule counting (SMC™) technology (RUO, Erenna® Immunoassay System, Singulex Inc., Alameda, CA, USA). Research assays of MR-proADM, galectin-3, and ST2 were developed by Alere, and have not been standardized to the commercialized assays used in research or in clinical use. The extent to which each Alere assay correlates with the commercial assay is not fully characterized. Assay information included inter-assay coefficient of variation are provided in *supplementary table* 2. Estimated glomerular filtration rate (eGFR) was based on the simplified Modification of Diet in Renal Disease (MDRD) (15).

Outcome.

The primary outcome of this study was all-cause mortality and/or rehospitalization at 60 days' post admission. This outcome was chosen because of the relatively large event rate in comparison to the other outcomes in the PROTECT trial. A blinded clinical events committee adjudicated the outcome.

Statistical analysis

Continuous variables are presented as means ± standard deviations or medians with interquartile ranges. Categorical variables are presented as numbers or percentages. Intergroup differences were analyzed using Students' t-test, Mann-Whitney-U test, Kruskal-Wallis test, Analysis of Variance (ANOVA) or chi2-test where appropriate.

To correct for multiple comparisons, principal component (PC) analysis was performed with

HFrEF and HFpEF as categorical variables, using an established method described elsewhere (16). A total of 27 PCs cumulatively explained >95% of the variation observed in the dataset when comparing HFrEF and HFpEF (supplementary figures 1 & 2). The corrected significance level for multiple testing was thus set at P <0.05/27. Following this, a spearman's rank correlation coefficient was calculated for each possible biomarker pair in the HFrEF cohort of patients and the procedure was repeated for HFpEF and HFmrEF. This resulted in three sets of R-values with associated p-values for HFrEF, HFmrEF and HFpEF. To adjust for multiple testing, only those correlations passing the adjusted p-value cut-off calculated from the PC-Analysis (PCA) were deemed statistically significant and subsequently retained. These significant correlation coefficients for HFrEF, HFmrEF and HFpEF were then graphically displayed as heatmaps with associated disease domains for all biomarkers. Network analysis was performed to analyze associations between biomarkers in HFrEF, HFmrEF and HFpEF. Subsequently, all significant associations found within HFrEF, HFmrEF and HFpEF were separately depicted as circular networks, consisting of nodes (biomarkers) and edges (associations). In each network, the size and color of the nodes reflect the clustering coefficient of each biomarker, while the thickness of the lines (edges) represent the strength of the inter-biomarker associations (determined by spearman's rank coefficient R values).

To study the possible differential relationship with outcome of biomarkers, a univariable interaction test was performed between LVEF and the biomarker levels at admission or a change in biomarker levels between admission and the first 24h. Following this, a multivariable interaction test was performed correcting for a risk engine containing 8 variables, specifically designed for this cohort (17). These variables include age, previous HF hospitalizations, peripheral edema, systolic blood pressure, serum sodium, urea, creatinine and albumin levels at admission. Univariable and multivariable associations of biomarkers with outcome were tested using Cox regression analysis; due to the exploratory nature of these analyses, a p-value of <0.05 was deemed statistically significant for the interaction test.

All tests were performed two-sided and a p-value of <0.05 was considered statistically

significant. All statistical analyses were performed using STATA version 11.0 (StataCorp LP, College station, Texas, USA) and R version 3.2.4.

Results.

Baseline characteristics

Baseline characteristics are presented in *table 2*. Patients with HFmrEF were older than HFrEF patients, but younger than HFpEF (71 vs. 68 and 74 years respectively, P-value for trend <0.001). With increasing LVEF, the percentage of female patients, BMI, systolic blood pressure and diastolic blood pressure was higher ($P_{\text{-trend}}$ <0.05). We observed less mitral regurgitation, less previous HF hospitalizations during the past year, and less ischemic heart disease and myocardial infarction with increasing LVEF ($P_{\text{-trend}}$ all <0.001). Median time since the previous HF hospitalization was 52 days and did not differ between HFrEF; HFmrEF and HFpEF (p = 0.776). In contrast, a history of hypertension ($P_{\text{-trend}}$ <0.001) and atrial fibrillation ($P_{\text{-trend}}$ 0.014) was found more often with increasing LVEF. A direct comparison between HFmrEF - HFrEF and HFmrEF - HFpEF confirms these results (*supplementary tables 3 & 4*).

Biomarker levels.

Biomarker levels at admission are presented in *table 3.* With increasing LVEF, we found increasing levels of CRP, NGAL, KIM-1 and platelet count and decreasing levels of GDF-15, BNP, Troponin-I, RBC, hemoglobin and endothelin-1. After correction for multiple comparisons, the up- or down sloping trend remained significant for BNP, KIM-1, RBC and hemoglobin. When examining a change of biomarkers from admission to 24-hours, troponin-I increased more in patients with HFrEF than in patients with HFmrEF and HFpEF, however significance was lost after correction for multiple comparisons (*supplementary table 5*). No significant interaction was found

between the study drug and LVEF for biomarkers that significantly differed between HFrEF; HFmrEF and HFpEF, also no significant interactions were observed between timing of echocardiography and LVEF for biomarker levels ($p_{\text{-interaction}}$ all >0.1).

Network analysis.

Heatmaps of biomarker associations are available in *supplementary figures 3-5.* The results of Network analysis are shown in *figure 1-3.* At admission, network analysis in HFrEF showed Troponin-I, BNP and PSAP-B to be a hub. A biomarker which is a hub has a high clustering coefficient. A high clustering coefficient suggests a certain centrality of the biomarker within the network, where a large number of the biomarker interactions are mediated through the hub. In HFpEF, angiogenin, hemoglobin, galectin-3 as well as d-dimer were hubs. Compared to HFrEF, BNP is only moderately associated with other biomarkers in HFpEF at admission. Interestingly, in HFmrEF, hemoglobin, RBC, endothelin-1 as well as BNP and galectin-3 were clear hubs at admission. After 24hrs interactions of biomarkers in patients with HFrEF were mainly associated with BNP and endothelin-1. In comparison, after 24hrs, biomarkers in HFpEF were mainly associated with inflammation markers pentraxin-3 and RAGE, as well as with remodeling marker osteopontin, angiogenesis marker angiogenin, hematopoiesis markers hemoglobin and red blood cell count as well as renal function marker NGAL. Interestingly, BNP remains a small hub in HFpEF. In HFmrEF, after 24hrs, the association between BNP and other biomarkers became very limited. Furthermore, remodeling marker galectin-3 and inflammation marker RAGE were continuous hubs at admission through the first 24hrs.

Biomarker levels and outcome.

Associations of biomarkers levels at admission with outcome are shown in *supplementary tables 6* Remodeling markers syndecan-1 (p = 0.047) and galectin-3 (p = 0.024) showed a significant

interaction for the primary outcome. Here, syndecan-1 showed a significant association with outcome in HFmrEF and HFpEF, but not in HFrEF. Also, galectin-3 showed significant predictive value in HFpEF, but not in HFmrEF and HFrEF.

The associations with outcome of a change of biomarker levels within the first 24 hours is show in *supplementary table 7*. A significant multivariable interaction was found for the inflammation biomarkers pentraxin-3 (p = 0.025), RAGE (p = 0.037), TNF-R1a (p = 0.004), oxidative stress marker MPO (p = 0.017) and the endothelial function marker proADM (p = 0.016) as well as arteriosclerosis marker LTBR (p = 0.009). Following multivariable correction, pentraxin-3 was more predictive in HFmrEF and HFpEF, but not in HFrEF. A change in levels of TNF-R1a, MPO and LTBR were related to outcome in HFpEF, but not in HFrEF and HFmrEF. Interestingly, a change of endothelial function marker pro-ADM only had predictive power in HFmrEF, but not in HFrEF and HFpEF (*supplementary table 7*).

Discussion.

This study demonstrates differential biomarker profiles between AHF patients with HFrEF, HFmrEF and HFpEF. Network analysis showed that in HFmrEF, interaction between biomarkers were associated with BNP, galectin-3 and endothelin-1. In contrast, interactions between biomarkers in HFrEF were mostly associated with BNP, KIM-1 and Troponin-I, while in HFpEF, biomarkers associated with inflammation and endothelial function played a central role. Both in terms of clinical characteristics and biomarker profiles, patients with HFmrEF were in between HFpEF and HFrEF. Biomarkers profiles of HFmrEF, HFpEF and HFrEF remained relatively stable throughout the first 24h post hospital admission. With regard to outcome, markers of inflammation showed independent predictive value in HFmrEF and HFpEF, but not in HFrEF. Levels of remodeling markers syndecan-1 and galectin-3 showed predictive value in HFmrEF and HFpEF, but not in HFrEF. Of note, pro-ADM showed predictive value in HFmrEF, but not in HFrEF and HFpEF.

Biomarker levels of patients with HFmrEF were between HFrEF and HFpEF. HFrEF patients had higher levels of biomarkers related to cardiac stretch and hematopoiesis. Network analysis showed an inter-association between biomarkers related to inflammation and cardiac stretch in HFmrEF. In HFpEF, associations related to inflammation and BNP only played a very marginal role in associations between biomarkers. In HFrEF, BNP had a more prominent role in network analyses both at admission and after 24h. In HFmrEF, a mix of associations between cardiac stretch and inflammation was observed. In an earlier publication in a chronic HF setting, associations between inflammation markers were seen in HFpEF, while in HFrEF associations were found between cardiac stretch markers (10). Indeed, also in this study, network analysis revealed patterns, which were previously unknown in HFrEF and HFpEF. Biomarkers in the intermediate group were more related to HFpEF than to HFrEF in this sub-analysis of the TIME-CHF trial (10). This could potentially be explained by the difference in inclusion criteria, where for the PROTECT trial a minimum NT-proBNP above >2000 pg/mL had to be present at admission, while this was not required for the TIME-CHF trial (18). HFpEF patients are known to have lower BNP and NT-proBNP levels compared to HFrEF, which could explain why the proportion of HFpEF patients in the PROTECT trial is lower (7).

Remodeling marker syndecan-1 had predictive value in HFmrEF and HFpEF, but not in HFrEF. This was previously shown in a stable HF setting, where syndecan-1 had predictive value in HFpEF but not in HFrEF (5). In an earlier publication about syndecan-1, HFpEF was defined at LVEF>40%, suggesting that syndecan-1 also in a chronic setting provides predictive value in both HFmrEF and HFpEF. Galectin-3 only showed predictive value in HFpEF, but not in HFrEF and HFmrEF, in line with an earlier publication (19). Furthermore, a change in levels of inflammation markers pentraxin-3 and TNF-R1a were predictive in HFpEF, but not in HFrEF. The role of pentraxin-3 in HFpEF is readily known (20). In earlier reports, circulating TNF-R1a levels predicted incident cardiovascular disease, including HF (21). In a particular study addressing chronic HF, TNF-R1 was the strongest predictor of long-term mortality (22). Higher levels of TNF-

304 R were previously reported in HFpEF patients (23). Levels of MPO were previously correlated
305 with NYHA stage and diastolic HF and is considered to be both a marker of inflammation and
306 oxidative stress (24, 25). A change in levels of MPO was predictive in HFpEF, but not in HFmrEF
307 and HFrEF. LTBR is a member of the tumor necrosis factor family (26, 27). Activation of LTBR
308 results in lymphocyte recruitment and is associated with inflammatory responses in atherosclerosis
309 (26, 28). No data is available on predictive value in HF; and this is the first study reporting the
310 differential involvement in predicting outcome in AHF patients with HFrEF, HFmrEF and HFpEF.
311 Of note, TNF-R1a and LTBR are members of the TNF family of cytokines, suggesting a possible
312 involvement of this family of proteins. Members of the TNF-alpha super family are involved in
313 nitric oxide handling, which is considered a key mechanism in HFpEF. Whether other members of
314 the TNF-alpha superfamily have a significant role in the pathophysiology of HFpEF needs to be
315 explored further.

316 The clinical implications of this study are fourfold. First of all, both the clinical and
317 biomarker profiles of patients with HFmrEF were in between of HFrEF and HFpEF. This suggests
318 that HFmrEF is a mix of patients similar to both HFrEF and HFpEF. There could be a considerable
319 number of patients among HFmrEF who are closer to HFrEF and might benefit from existing HF-
320 guideline directed therapy. Previously, large HF trials had either excluded or embedded HFmrEF
321 within the HFpEF group (1). Future studies should distinguish which HFmrEF patients are closer to
322 HFrEF and which are closer to HFpEF. Biomarkers could aid in recognizing patients with HFmrEF
323 that are closer to HFrEF. These patients are likely characterized by high NT-proBNP and high
324 cardiac damage markers, while having lower levels of inflammation markers compared to HFpEF
325 patients. These patients could subsequently benefit from guideline-directed therapy and can
326 possibly be included in future HF trials with HFrEF patients. Secondly, patients with HFpEF have a
327 distinct biomarker profile from those with HFrEF, with patients with HFpEF having lower levels of
328 cardiac stretch markers. Also, inflammation related biomarkers had more predictive value in HFpEF
329 and HFmrEF than in HFrEF. Thirdly, overall biomarker profiles stay relatively stable in both

HFrEF, HFmrEF and HFpEF during hospitalization, in which biomarker associations are more angiogenesis and inflammation related in HFpEF, cardiac stretch related in HFrEF and both cardiac stretch and inflammation related in HFmrEF.

Limitations of the study

This study is a retrospective post-hoc analysis, which is accompanied by a possible selection bias. Not all patients had complete biomarker data available at admission and after 24h, creating a potential selection bias. Also, despite the large number of biomarker available, the choice for biomarkers was restricted by limited sample availability. It also needs to be emphasized that this is a data driven approach and causality cannot be proven. Results of this study need to be validated in a different population. Additionally, some echocardiographic measurements were performed 6 months prior to admission. This did not seem to influence biomarker levels in HFrEF; HFmrEF and HFpEF, however we could not correct for this in network analysis. Differences with regard to outcome prediction should only be interpreted in the context of pathophysiological differences between HFrEF, HFmrEF and HFpEF and not with respect to possible clinical utility (10). For the latter, the relatively low number of events confounds the results with regard to predictive value. This was especially true for other outcomes (e.g., 30-day mortality) in the PROTECT trial, for which the number of events was even lower than the outcome used, making useful statistics on these outcomes not possible. Confirmation of the differential predictive value found is needed in more inclusive independent trials with larger number of events and HFmrEF and HFpEF patients.

Conclusions.

Clinical characteristics and biomarker profiles of patients with HFmrEF are between patients with HFrEF and HFpEF, suggesting HFmrEF to be a heterogeneous group. Biomarker associations in HFpEF were mostly inflammation based, whilst being more cardiac stretch based in HFrEF. Biomarkers related to inflammation and cardiac remodeling had predictive value in HFmrEF and

HFpEF, but not in HFrEF. These data suggest that patients with HFmrEF are a mix of HFrEF and HFpEF patients. Distinguishing HFmrEF patients closer to HFrEF could have important therapeutic consequences for this group.

Competency in medical knowledge

Differences between AHF patients with HFmrEF, HFrEF and HFpEF have not been well characterized. Results from this study suggest that AHF patients with HFpEF have a significantly different biomarker profile from patients with HFrEF. Herein, we found that inflammation plays a larger role in patients with HFpEF compared to HFrEF. Secondly, patients with HFmrEF are in between patients with HFpEF and HFrEF. This suggests that these patients should be carefully considered when treating according to guidelines, since some of them might be closer to HFrEF and some might be closer to HFpEF. Lastly, a change in inflammation biomarker levels might hold prognostic value for patients with HFpEF and HFmrEF.

Translational outlook.

Biomarker based characterization of patient populations might help to identify novel treatment targets as well as decipher disease heterogeneity and underlying differences in pathophysiology. While biomarker based clinical studies can be considered a crude tool, it can be the first step in identifying novel disease entities and pathophysiological targets. Findings from biomarkers based studies, including this one, should be validated in an experimental setting.

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Conflict of interest

Dr. Cleland was on the Steering Committee for the PROTECT trial; served on the advisory board for MSD; and received payments for both. Dr. O'Connor is a consultant to Merck & Co., Inc. Dr. Ponikowski has received honoraria from Merck & Co., Inc; Dr. Davison and Dr. Cotter are employees of Momentum Research Inc, which was contracted to perform work on the project by Merck & Co., Inc. Dr. Metra have received honoraria and reimbursements from NovaCardia, sponsors of the study, and Merck & Co., Inc. Dr. Givertz has received institutional research support and served on a scientific Advisory Board for Merck & Co., Inc. Dr. Teerlink has received research funds and = consulting fees from Merck & Co., Inc. Dr. Bloomfield is an employee of Merck & Co., Inc. Dr. Dittrich served as a consultant to Merck & Co., Inc. Dr. Voors has received speaker and consultancy fees from Merck & Co., Inc was on the Steering Committee for the PROTECT trial. He also received research support from Alere, Singulex, and Sphingotec. All other authors have reported that they have no conflict of interest to declare

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Figure legends:

Figure 1: Network analysis illustrating correlative associations between biomarkers for HFrEF at admission (a) and 24 hours (b). The size and color of each node (hub) depicts the clustering coefficient where a large node reflects a high clustering coefficient. In addition, a color closer to blue depicts a higher clustering coefficient, while a color closer to red is associated with a lower clustering coefficient. Furthermore, the thickness and color of the lines connecting biomarkers to each other reflect the strength of the inter-biomarker associations.

Figure 2: Network analysis illustrating correlative associations between biomarkers for HFmrEF at admission (a) and 24 hours (b). The size and color of each node (hub) depicts the clustering coefficient where a large node reflects a high clustering coefficient. In addition, a color closer to blue depicts a higher clustering coefficient, while a color closer to red is associated with a lower clustering coefficient. Furthermore, the thickness and color of the lines connecting biomarkers to each other reflect the strength of the inter-biomarker associations.

Figure 3: Network analysis illustrating correlative associations between biomarkers for HFpEF at admission (a) and 24 hours (b). The size and color of each node (hub) depicts the clustering coefficient where a large node reflects a high clustering coefficient. In addition, a color closer to blue depicts a higher clustering coefficient, while a color closer to red is associated with a lower clustering coefficient. Furthermore, the thickness and color of the lines connecting biomarkers to each other reflect the strength of the inter-biomarker associations.

Table 1: Biomarker classification

	Inflammation/ Immune system	Remodeling	Oxidative stress	Cardiomyocyte stress/injury	Endothelial function	Atherosclerosis	Angiogenesis	Renal function	Metabolic markers	Hematopoiesis	Other
Angiogenin							X				
BNP				X							
BUN								X	X		
Creatinine								X			
CRP	X					X					
D-Dimer	X										X
Endothelin-1	X		X	X							X
ESAM	X					X	X				
Galectin-3	X	X						X			
GDF-15	X	X	X			X					
Hemoglobin										X	
Interleukin-6	X										
KIM-1								X			
LTBR	X					X					
Mesothelin											X
MPO	X		X								
Neuropilin					X		X				X
NGAL	X							X			
NT-proCNP					X						
Osteopontin	X	X				X	X				
PCT	X										
Pentraxin-3	X										

Periostin		X								X
PIGR	X									X
Platelet count									X	X
proADM					X					
PSAP-B			X							X
RAGE	X					X				
RBC count										
ST-2	X	X	X	X			X			
Syndecan-1	X	X								
TNF-R1a	X									
Troponin-I				X						
TROY	X	X								
VEGFR							X			
WAP4C	X									X
WBC count	X								X	

Abbreviations: CRP, C-reactive protein; ESAM, endothelial cell-selective adhesion molecule; ET-1, endothelin-1; GDF-15, growth differentiation factor 15; HFpEF, heart failure with a preserved ejection fraction; HFrEF, heart failure with a reduced ejection fraction; IL-6, interleukin-6; KIM-1, kidney injury molecule 1; LTBR, lymphotoxin beta receptor; NGAL, neutrophil Gelatinase-associated Lipocalin; NT-proBNP, N-terminal pro-brain natriuretic peptide; NT-proCNP, N-terminal pro-C-type natriuretic peptide; PCT, procalcitonin; PIGR, Polymeric immunoglobulin receptor; proADM, pro-adrenomedulin; PSAP-B, Prosaposin B; RAGE, Receptor for advanced glycation end product; RBC, red blood cell count; ST-2, Soluble ST-2; TNF-R1, tumor necrosis factor alpha receptor 1; VEGFR-1, vascular endothelial growth receptor 1A, WAP-4C, WAP Four-Disulphide Core Domain Protein HE; WBC, white blood cell count.

Table 2: Baseline characteristics.

	HFrEF	**HFmrEF**	**HFpEF**	**p-value**	**p-value trend**
N	607	128	108		
Demographics					
Age, years, mean ± SD	68.0 ± 12.0	70.7 ± 11.3	74.4 ± 10.1	**<0.001**	**<0.001**
Female sex, n (%)	137 (22.6%)	76 (59.4%)	57 (52.8%)	**<0.001**	**<0.001**

BMI, kg/m2, mean ± SD	28.1 ± 5.7	29.0 ± 7.1	29.6 ± 7.0	**0.029**	**0.027**
eGFR, mL/min/1.73 m2, mean ± SD	48.4 ± 19.5	48.1 ± 18.7	47.0 ± 21.5	0.800	0.353
NYHA class, n (%)				0.290	0.186
I/II	90 (15.6%)	27 (21.8%)	16 (16.5%)		
III	329 (57.1%)	64 (51.6%)	61 (62.9%)		
IV	157 (27.3%)	33 (26.6%)	20 (20.6%)		
LVEF, median (IQR)	25 (20, 30)	42 (40, 45)	56 (50, 60)	**<0.001**	**<0.001**
Systolic BP, mmHg, mean ± SD	119.3 ± 17.2	127.1 ± 16.0	134.2 ± 17.2	**<0.001**	**<0.001**
Diastolic BP, mmHg, mean ± SD	72.5 ± 11.9	73.5 ± 12.2	74.7 ± 13.5	0.190	**0.027**
Heart rate, b.p.m. mean ± SD	80.3 ± 14.9	78.5 ± 15.6	79.0 ± 16.8	0.410	0.588
Rolofylline, n(%)	406(66.9%)	90 (70.3%)	70 (64.8)	0.648	0.920
Medical history, n (%)					
Mitral regurgitation,	298 (49.2%)	40 (31.3%)	28 (26.2%)	**<0.001**	**<0.001**
Heart failure (HF),	578 (95.2%)	124 (96.9%)	97 (89.8%)	**0.034**	0.078
Hospitalization for HF previous year	356 (58.6%)	70 (54.7%)	49 (45.4%)	**0.034**	**0.011**
HF hospitalizations, median (IQR)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	1.0 (1.0, 2.0)	0.560	0.278
Ischemic heart disease	434 (71.7%)	86 (67.2%)	58 (53.7%)	**<0.001**	**<0.001**
Myocardial infarction	351 (58.0%)	57 (44.5%)	25 (23.4%)	**<0.001**	**<0.001**
Hypertension	425 (70.0%)	112 (87.5%)	95 (88.0%)	**<0.001**	**<0.001**
Stroke or PVD	117 (19.3%)	25 (19.5%)	24 (22.2%)	0.780	0.519
COPD or asthma	146 (24.2%)	15 (11.7%)	26 (24.1%)	**0.008**	0.261
Diabetes mellitus	275 (45.4%)	63 (49.2%)	42 (38.9%)	0.280	0.419

Atrial Fibrillation/Flutter	305 (50.5%)	69 (53.9%)	71 (65.7%)	**0.014**	**0.005**
Medication prior to admission, n (%)					
Beta-blockers	485 (80.0%)	93 (72.7%)	85 (78.7%)	0.180	0.348
ACE-I/ARB	455 (75.1%)	91 (71.1%)	82 (75.9%)	0.610	0.86
MRA	311 (51.3%)	49 (38.3%)	32 (29.6%)	**<0.001**	**<0.001**
Digoxin	170 (28.1%)	35 (27.3%)	23 (21.3%)	0.350	0.182
Nitrates	142 (23.5%)	28 (21.9%)	26 (24.1%)	0.910	0.984
CCBs	41 (6.8%)	22 (17.2%)	28 (25.9%)	**<0.001**	**<0.001**
Presenting signs & symptoms, n (%)					
Orthopnea	489 (82.5%)	105 (83.3%)	85 (79.4%)	0.710	0.564
Dyspnea at rest (NYHA IV)	323 (55.6%)	71 (57.7%)	56 (54.4%)	0.870	0.963
Angina pectoris	117 (19.3%)	31 (24.2%)	21 (19.6%)	0.450	0.602
Edema	155 (25.6%)	30 (23.4%)	34 (31.5%)	0.340	0.349
JVP	251 (45.6%)	52 (46.8%)	39 (39.4%)	0.480	0.362

Abbreviations: ACE-I, ACE-inhibitors; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; CCB, calcium channel blocker; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; HFpEF, heart failure with a preserved ejection fraction; HFmrEF, heart failure with a reduced ejection fraction; IQR, inter-quartile range; JVP, Increased jugular venous pressure; LVEF, left ventricular ejection fraction; MRA, mineral receptor antagonist; NYHA, New York heart association; PVD, peripheral vascular disease; SD, standard deviation

Table 3: Biomarker levels at admission.

	HFrEF	HFmrEF	HFpEF	p-value	p-value*	p-value for trend	p-value for trend*
N	607	128	108				
Inflammation/Immune system							
WBC (x10^9/L)	7.6 (6.2, 9.2)	7.3 (6.3, 8.8)	7.4 (6.1, 10.0)	0.560	1.000	0.997	1.000
CRP (ng/ml)	13350.1 (7116.7, 28145.4)	12937.1 (7483.5, 26490.9)	18801.0 (10274.2, 31983.5)	**0.043**	1.000	**0.025**	0.675
GDF-15 (ng/ml)	4.9 (3.1, 6.3)	4.1 (2.9, 6.3)	4.5 (3.0, 6.3)	**0.034**	0.924	**0.022**	0.594
PCT (ng/ml)	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.820	1.000	0.603	1.000
Pentraxin-3 (ng/ml)	4.5 (3.0, 7.0)	3.8 (2.5, 7.3)	3.9 (2.8, 6.3)	0.074	1.000	0.057	1.000
RAGE (ng/ml)	5.1 (3.7, 6.8)	4.8 (3.5, 6.5)	4.7 (3.6, 6.6)	0.500	1.000	0.245	1.000
TNF-R1a (ng/ml)	3.3 (2.2, 4.8)	3.0 (2.1, 4.6)	3.6 (2.3, 5.2)	0.120	1.000	0.325	1.000
TROY (ng/ml)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.540	1.000	0.408	1.000
Interleukin 6 (pg/ml)	11.0 (6.0, 21.2)	10.2 (6.2, 15.7)	13.3 (6.6, 22.3)	0.400	1.000	0.764	1.000
Oxidative stress							
MPO (ng/ml)	32.7 (17.8, 67.1)	35.3 (16.1, 78.2)	32.3 (16.6, 66.7)	0.950	1.000	0.999	1.000
Remodeling							
Syndecan-1 (ng/ml)	8.5 (7.2, 10.6)	8.1 (6.9, 9.7)	8.8 (7.1, 10.8)	0.093	1.000	0.442	1.000
Periostin (ng/ml)	5.8 (3.4, 9.7)	5.7 (3.4, 8.8)	5.4 (3.1, 8.5)	0.440	1.000	0.198	1.000
Galectin-3 (ng/ml)	36.2 (27.0, 48.5)	35.4 (27.3, 48.7)	40.1 (30.3, 53.1)	**0.039**	1.000	0.300	1.000
Osteopontin (ng/ml)	112.1 (78.6, 172.4)	112.7 (84.2, 151.3)	112.9 (71.3, 179.9)	0.920	1.000	0.687	1.000
ST-2 (ng/ml)	3.4 (1.0, 8.7)	2.8 (0.9, 6.6)	3.9 (1.2, 7.2)	0.150	1.000	0.565	1.000
Cardiomyocyte stress/injury							
BNP (pg/ml)	520.9 (289.5, 877.9)	397.3 (214.8, 667.9)	300.1 (221.7, 600.9)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Troponin I (pg/ml)	11.9 (6.0, 23.6)	10.9 (6.1, 23.3)	8.4 (4.7, 18.5)	0.0515	1.000	**0.026**	0.702
Angiogenesis/Endothelial function							
VEGFR (ng/ml)	0.4 (0.3, 0.6)	0.4 (0.2, 0.5)	0.3 (0.2, 0.5)	0.036	0.976	0.012	0.324
Angiogenin (ng/ml)	1856.6 (1245.7, 2723.7)	2080.2 (1353.0, 2893.4)	1755.9 (1333.6, 2917.9)	0.160	1.000	0.639	1.000
Neuropilin (ng/ml)	12.9 (8.3, 18.3)	11.2 (8.1, 15.4)	12.2 (8.1, 17.0)	0.170	1.000	0.184	1.000
proADM (ng/ml)	2.9 (1.6, 5.0)	2.5 (1.5, 4.1)	2.8 (1.5, 5.3)	0.150	1.000	0.739	1.000

NTpro-CNP (ng/ml)	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	0.750	1.000	0.451	1.000
Atherosclerosis							
ESAM (ng/ml)	62.5 (56.4, 70.0)	61.7 (56.1, 68.3)	62.6 (57.5, 70.5)	0.440	1.000	0.872	1.000
LTBR (ng/ml)	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	0.5 (0.3, 0.6)	0.140	1.000	0.068	1.000
Renal function							
NGAL (ng/ml)	81.9 (54.4, 129.5)	76.8 (55.7, 143.9)	102.0 (62.9, 154.9)	**0.033**	0.883	**0.020**	1.000
KIM 1 (pg/ml)	269.4 (178.6, 462.9)	327.5 (218.2, 650.2)	351.2 (232.3, 585.7)	**0.001**	**0.021**	**<0.001**	**<0.001**
BUN (mg/dl)	31.0 (23.0, 44.0)	28.0 (21.0, 39.0)	30.0 (22.0, 41.0)	0.060	1.000	0.135	1.000
Hematopoiesis							
RBC ($x10^{12}$/L)	4.2 (3.8, 4.7)	4.2 (3.7, 4.6)	3.9 (3.5, 4.4)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Hemoglobin (g/dL)	12.6 (11.4, 13.8)	12.1 (10.8, 13.6)	11.6 (10.4, 12.6)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Other							
Endothelin 1 (pg/ml)	6.9 (5.2, 9.3)	6.3 (4.8, 8.0)	6.3 (4.2, 9.2)	**0.015**	0.402	**0.009**	0.243
D-Dimer (ng/ml)	155.2 (90.6, 340.3)	171.0 (90.6, 333.8)	176.0 (90.6, 338.6)	0.350	1.000	0.187	1.000
PIGR (ng/ml)	406.0 (262.5, 647.1)	379.9 (274.9, 604.5)	401.3 (256.3, 694.4)	0.880	1.000	0.815	1.000
PSAP-B (ng/ml)	40.6 (29.5, 55.2)	34.8 (26.6, 52.8)	36.3 (26.8, 56.7)	0.035	1.000	0.076	1.000
WAP4C (ng/ml)	28.8 (14.9, 55.0)	28.2 (13.8, 49.5)	28.5 (14.4, 59.6)	0.720	1.000	0.978	1.000
Mesothelin (ng/ml)	88.4 (75.2, 102.4)	85.4 (71.4, 96.6)	87.8 (77.4, 103.8)	0.097	1.000	0.443	1.000
Glucose (mg/dL)	126.0 (103.0, 159.0)	119.0 (97.0, 166.0)	121.0 (94.0, 159.0)	0.310	1.000	0.128	1.000
Platelet count ($x10^{9}$/L)	212.0 (165.0, 264.0)	215.0 (170.0, 287.0)	238.5 (190.0, 308.0)	**0.010**	0.279	**0.003**	0.081

Abbreviations: CRP, C-reactive protein; ESAM, endothelial cell-selective adhesion molecule; ET-1, endothelin-1; GDF-15, growth differentiation factor 15; HFpEF, heart failure with a preserved ejection fraction; HFrEF, heart failure with a reduced ejection fraction; IL-6, interleukin-6; KIM-1, kidney injury molecule 1; LTBR, lymphotoxin beta receptor; NGAL, neutrophil Gelatinase-associated Lipocalin; NT-proBNP, N-terminal pro-brain natriuretic peptide; NT-proCNP, N-terminal pro-C-type natriuretic peptide; PCT, procalcitonin; PIGR, Polymeric immunoglobulin receptor; proADM, pro-adrenomedulin; PSAP-B, Prosaposin B; RAGE, Receptor for advanced glycation end product; RBC, red blood cell count; ST-2, Soluble ST-2; TNF-R1a, tumor necrosis factor alpha receptor 1; VEGFR-1, vascular endothelial growth receptor 1A, WAP-4C, WAP Four-Disulphide Core Domain Protein HE; WBC, white blood cell count.

Figures.

Figure 1a

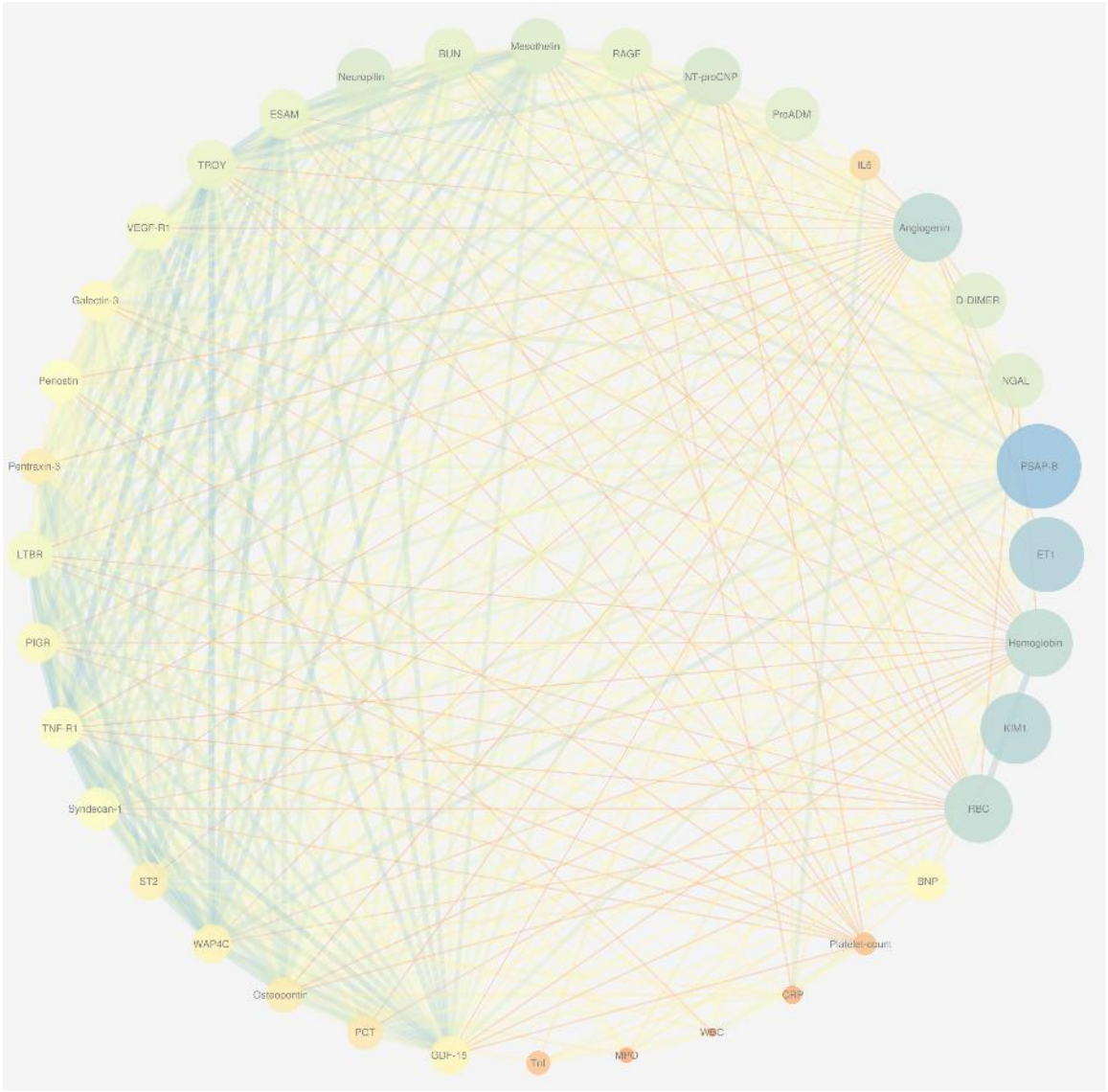

Figure 1b

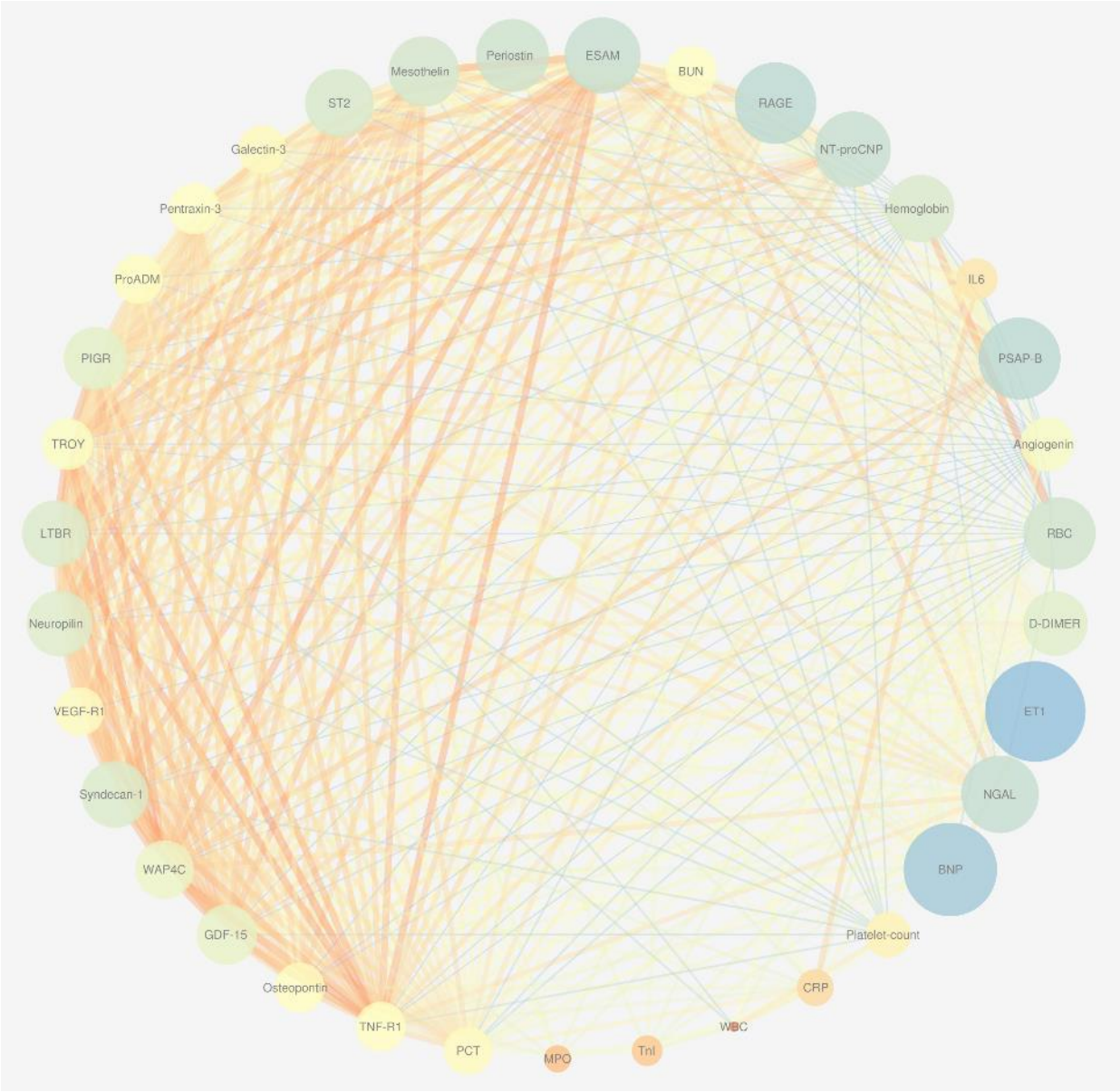
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Figure 2a

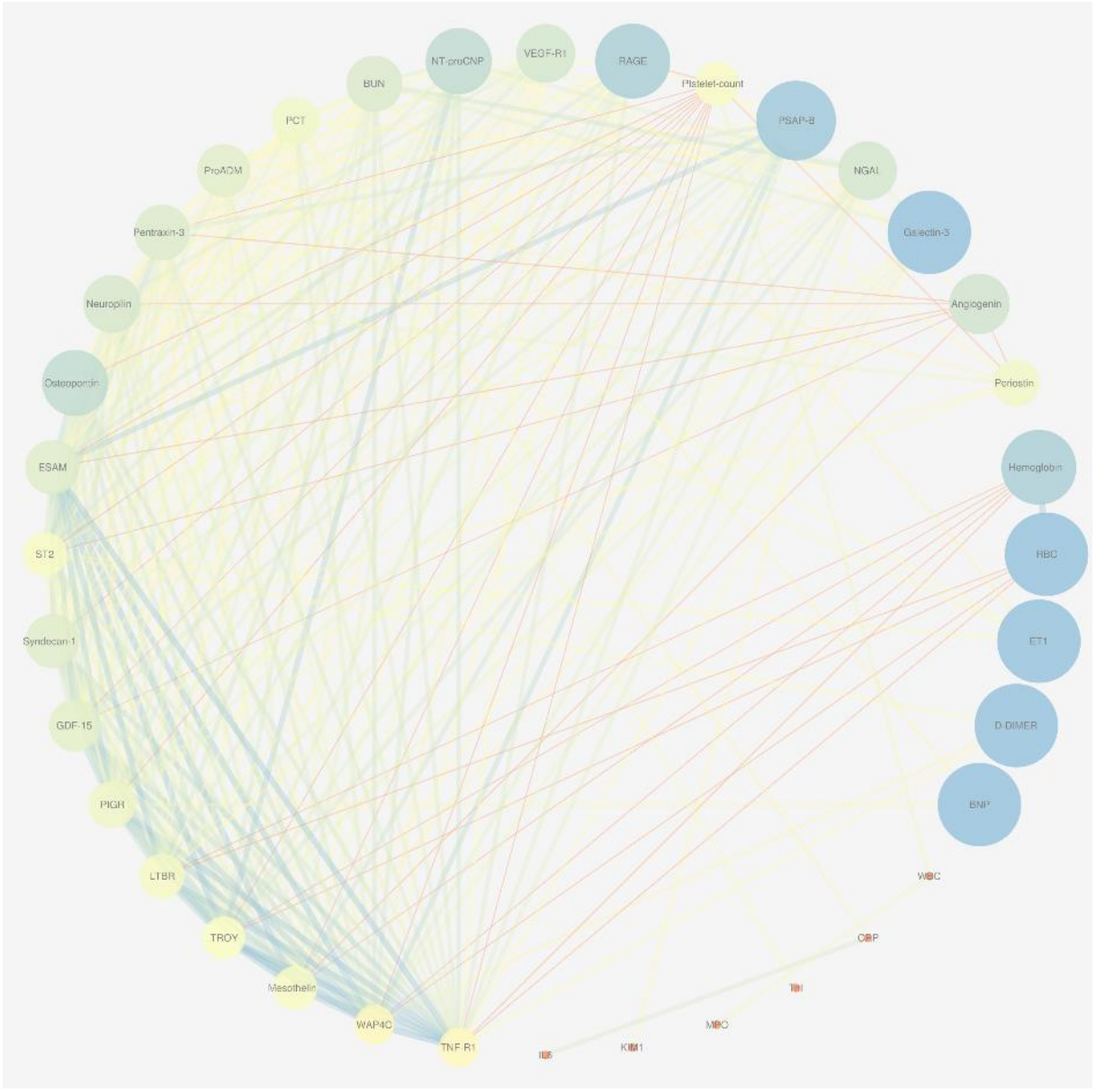
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Figure 2b

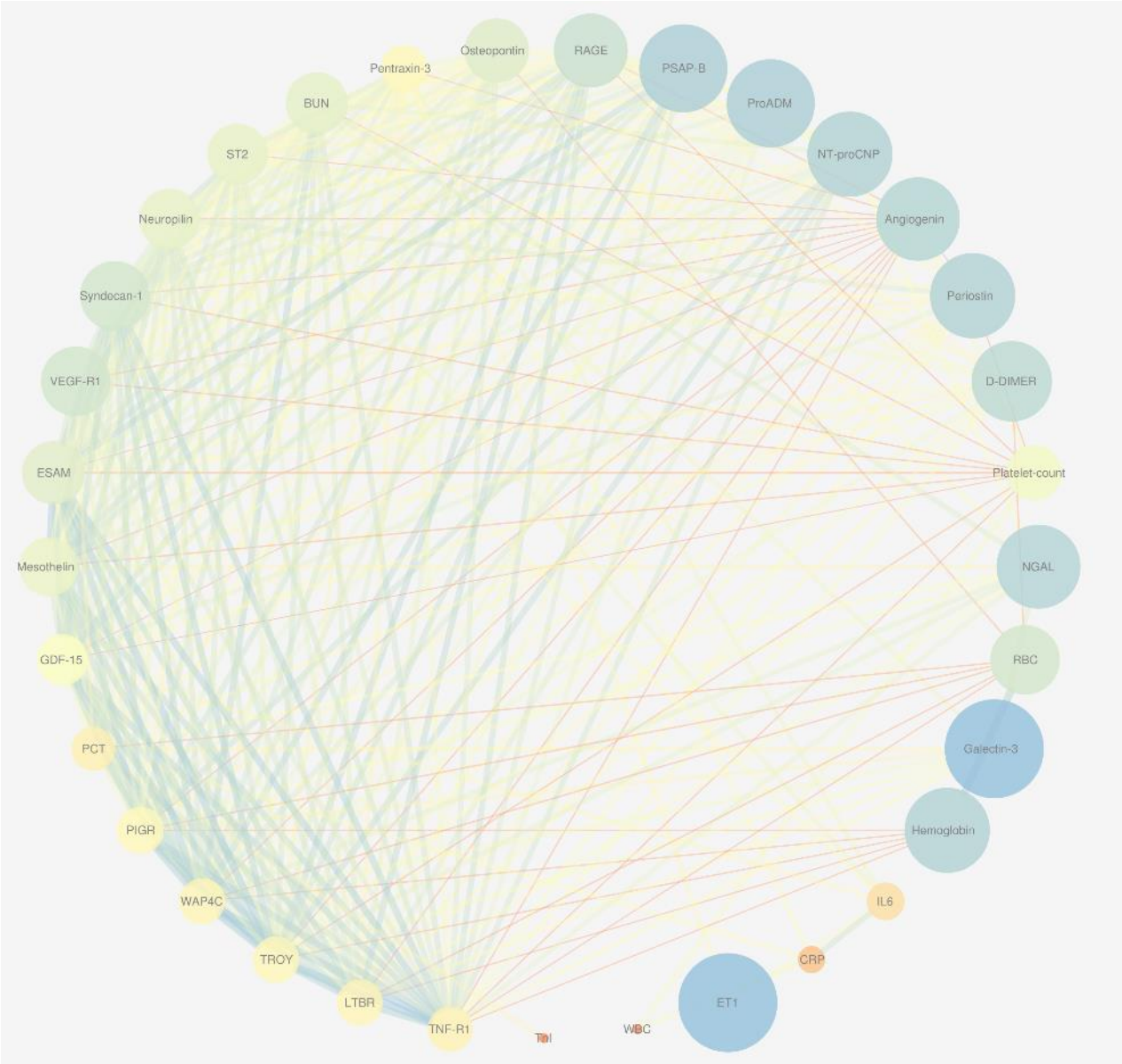
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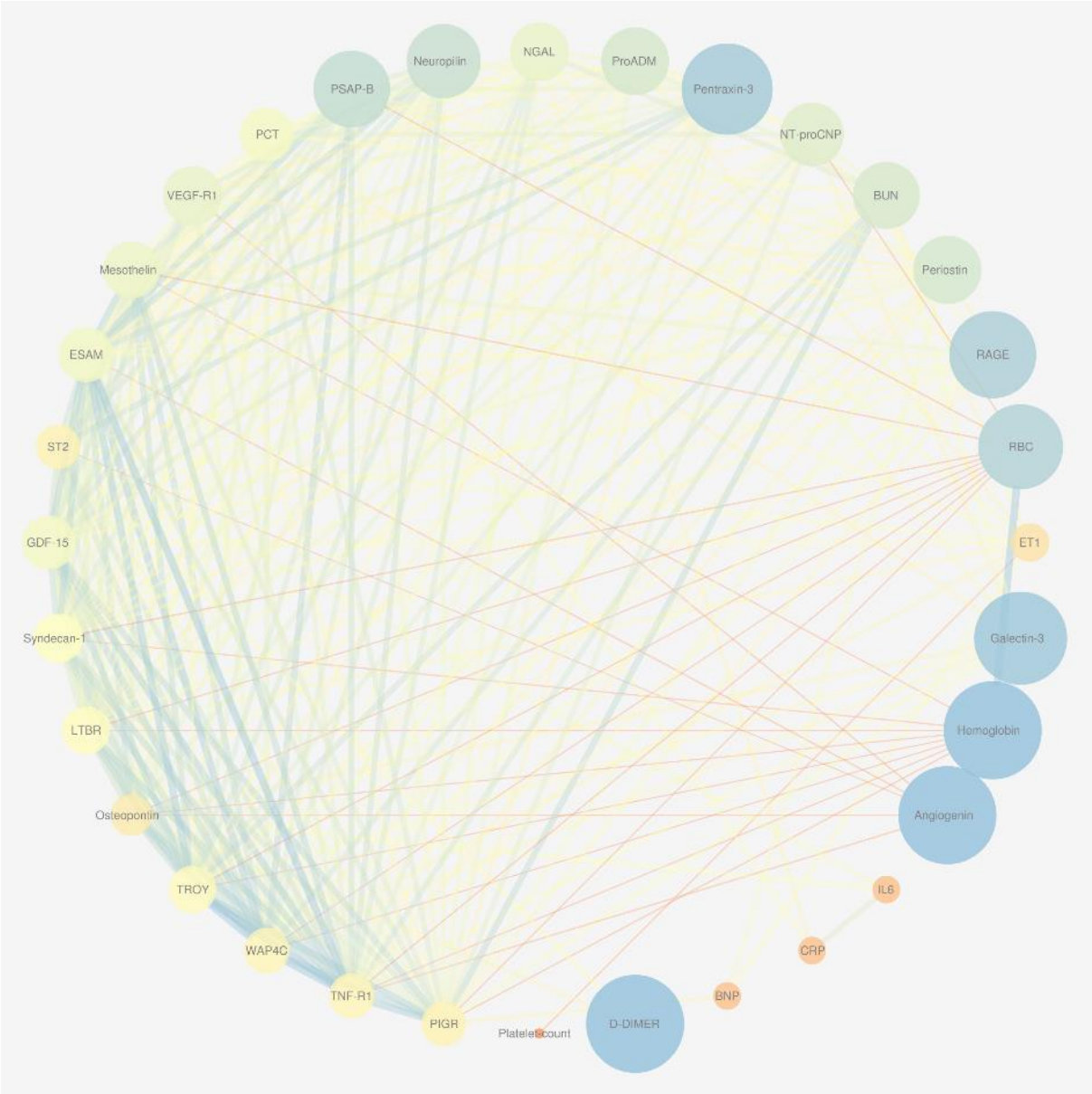
Figure 3a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ST2
RBC
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Galectin-3
LTBR
Hemoglobin
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CRP
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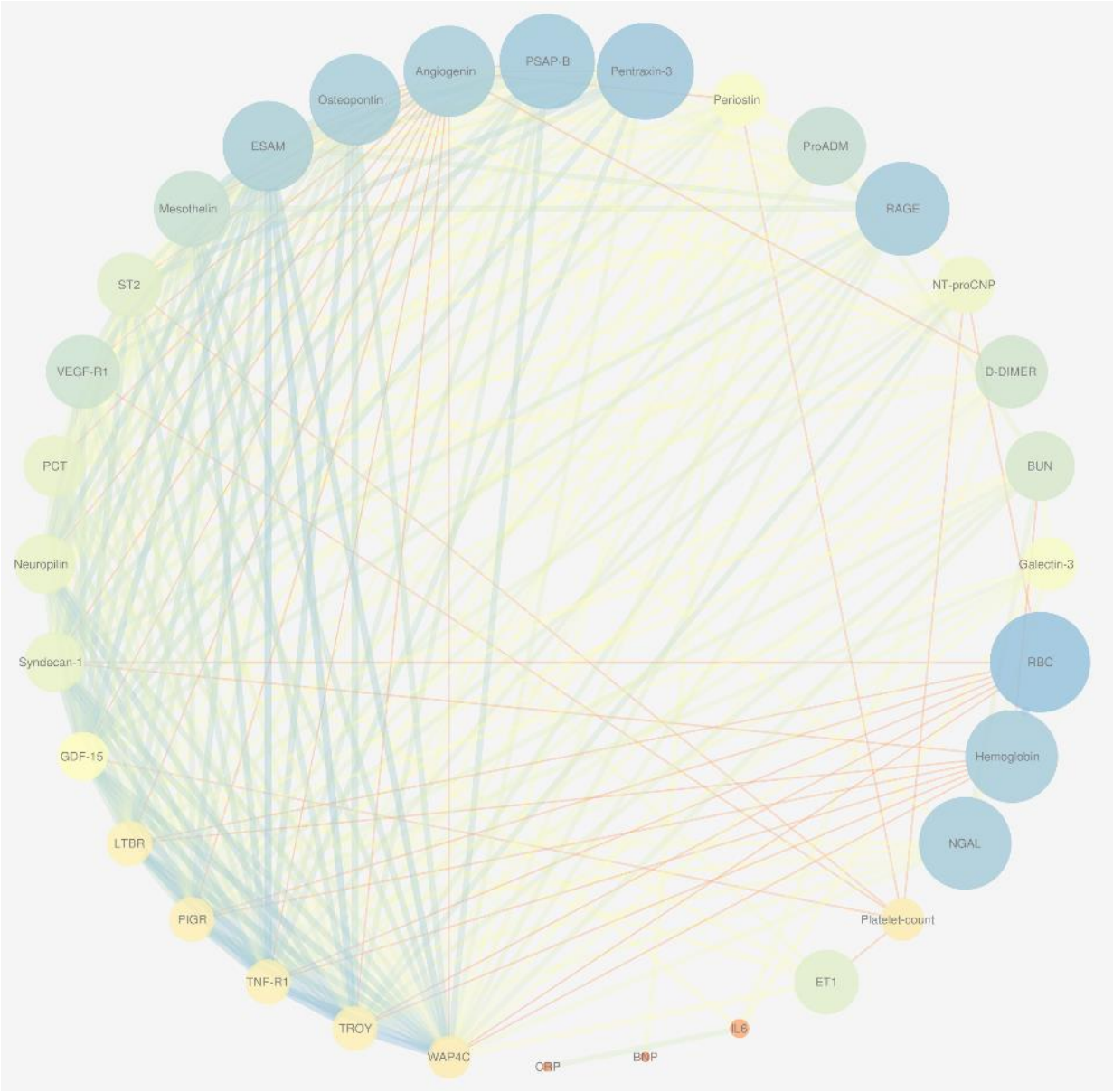
BNP
PIGR
D-DIMER
Platelet count

Figure 3b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RAGE
ST2
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VEGF-R1
D-DIMER
PCT
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