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A network analysis to compare biomarker profiles in patients with and without diabetes mellitus in acute heart failure

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Abstract

Aims

It is unclear whether distinct pathophysiologic processes are present among patients with acute heart failure (AHF), with and without diabetes. Network analysis of biomarkers may identify correlative associations which reflect different pathophysiologic pathways.

Methods and results

We analyzed a panel of 48 circulating biomarkers measured within 24 hours of admission for AHF in a subset of patients enrolled in the PROTECT trial. In patients with and without diabetes, we performed a network analysis to identify correlations between measured biomarkers. Compared to patients without diabetes (n=1111), those with diabetes (n=922) had higher prevalence of ischemic heart disease and traditional coronary risk factors. Patients with and without diabetes, after multivariable adjustment, had significantly different levels of biomarkers across a spectrum of pathophysiologic domains including inflammation (TNF-1 α , periostin), cardiomyocytes stretch (BNP), angiogenesis (VEGFR, angiogenin), and renal function (NGAL, KIM-1) (adjusted p-value <0.05). Among patients with diabetes, network analysis revealed that periostin strongly clustered with C-reactive protein and interleukin-6. Furthermore, renal markers (creatinine and NGAL) closely associated with potassium and glucose. These findings were not seen among patients without diabetes

Conclusion

Patients with AHF and diabetes, compared to those without diabetes, have distinct biomarker profiles. Network analysis suggests that cardiac remodeling, inflammation, and fibrosis are closely associated with each other in patients with diabetes; Furthermore, potassium levels may be sensitive to changes in renal function as reflected by the strong renal-potassium-

glucose correlation. These findings were not seen among patients without diabetes and may suggest distinct pathophysiologic processes among patients with diabetes.

Introduction

The prevalence of diabetes mellitus in the general population is 4-7%¹, and 32-44% in patients hospitalized for acute heart failure (AHF).²⁻⁵ Different underlying pathophysiologic processes including inflammation and fibrosis may be present among patients with diabetes compared to those without diabetes.^{6,7} These differences in pathophysiology may also be seen between patients with and without diabetes who have HF⁸⁻⁹; however, evidence to support this is limited. Network analysis is an analytic technique used to gain insights into a biological system by predicting how multiple genes or proteins associate together. This analytic technique has been extensively used in aging and cancer studies¹⁰⁻¹¹ to gain greater insights into underlying disease mechanisms. Network analyses of blood biomarkers have been previously used to explore pathophysiologic mechanisms in HF.^{12,13} Using network analysis, we can explore whether the underlying pathophysiologic mechanisms among patients with and without diabetes are different in the setting of AHF. Traditionally, individual biomarkers would be correlated to clinical characteristics and outcomes in an attempt to gain an understanding of a disease; however, multiple mechanisms are likely active among patients with AHF.¹⁴ Network analysis allows for multiple biomarkers - across a spectrum of pathophysiologic domains - to be assessed for correlations simultaneously, leading to greater insights into the pathophysiology of disease states. Using an extensive set of biomarkers measured in patients with and without diabetes admitted for AHF, this study aimed to evaluate (1) the differences in biomarker levels and (2) the patterns of inter-biomarkers correlations using network analysis.

Methods

Study design and procedures

The Placebo-controlled Randomized Study of the Selective A1 Adenosine Receptor

Antagonist Rolofylline for Patients Hospitalized with AHF and Volume Overload to Assess Treatment Effect on Congestion and Renal Function (PROTECT) trial, a multicenter, randomized, double-blind, placebo-controlled trial with neutral results, enrolled 2033 adult patients hospitalized for AHF.¹⁵ The main results of the study have been published previously.^{15,16} Key inclusion criteria included; persistent dyspnea at rest or with minimal activity, impaired renal function (an estimated creatinine clearance of 20 to 80 ml per minute with the use of the Cockcroft–Gault equation), a brain natriuretic peptide (BNP) level of 500 pg per milliliter or more or an N-terminal pro-brain natriuretic peptide (NT-proBNP) level of 2000 pg per milliliter or more, ongoing intravenous loop-diuretic therapy, and enrollment within 24 hours after admission. All patients provided informed consent for the study including assessment of biomarker. The study was conducted in compliance with the Declaration of Helsinki and was approved by all local Ethics Committees.

All routine laboratory values were assessed daily until discharge or day 6 (or discharge if earlier), and on days 7 and 14, as specified by the main study protocol. Additional biomarkers used in the present analysis were measured during baseline assessment. Full details of the biomarkers are described elsewhere.¹⁷ Briefly, a panel of novel and established biomarkers were measured by Alere Inc., San Diego, CA, USA in available frozen serum samples collected from each patient during baseline assessment. 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 (NT-proCNP), Osteopontin, procalcitonin (PCT), Pentraxin-3, Periostin,

Polymeric immunoglobulin receptor (PIGR), pro-adrenomedullin (proADM), prosaposin B (PSAP-B), Receptor for Advanced Glycation Endproducts (RAGE), soluble ST2, Syndecan-1, tumor necrosis factor alpha receptor 1 (TNF-R1a), Tumor necrosis factor receptor superfamily (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. Immunoassays for PCT, proADM, Galectin-3 and ST2 were developed by Alere. These research assays have not been standardized to the commercialized assays used in research or in clinical use and the extent to which each Alere assay correlates with the commercial assay is not fully characterized. Four additional biomarkers including Interleukin-6, Endothelin-1, Kidney injury marker -1 (KIM1), cardiac Troponin-I, and BNP were measured using a high sensitive single molecule counting (SMC™) technology (RUO, Erenna® Immunoassay System, Singulex Inc., Alameda, CA, USA). Glomerular filtration rate (GFR) was estimated using the simplified MDRD equation. Biomarker details are presented in supplementary appendix table 1. In addition to these biomarker, we used baseline sodium, potassium, chloride, creatinine, urea, uric acid, total cholesterol, triglycerides, red blood cell count, hematocrit, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in our network analyses. Approximately 250 microL of EDTA plasma was used to measure the 48 biomarkers. As this was a hypothesis generating analysis, we used all available biomarkers and routinely measured clinically biomarkers in our analysis

Study population

The primary study population comprised of 922 patients with diabetes and 1111 without diabetes. Biomarker data was available for 808 heart failure patients with DM and for 970 heart failure patients without diabetes. Baseline data on patients with and without biomarkers

have been previously reported.¹⁷ The definition of diabetes was ascertained through the electronic case report form and was based on patient reported history of diabetes and use of anti-diabetic drugs. Data on the type of diabetes (i.e. type 1 vs. type 2) or the degree of glycemic control (through HbA1c) were not collected.

Statistical analyses

Continuous variables are presented as mean \pm standard deviation or median (interquartile range) for normally and non-normally distributed values respectively. Student's t-tests and Wilcoxon tests were used to compare groups, as appropriate. A two-sided *P*-value < 0.05 was considered statistically significant. To assess for significant differences in biomarker levels after adjustment for co-morbidities and medication use in patients with and without diabetes, we conducted a multivariable linear regression analysis in which each biomarker was entered as a dependent variable and diabetes and potential confounders (age, sex, ischemic heart disease, peripheral vascular disease, estimated glomerular filtration rate, and angiotensin converting enzyme inhibitor [ACEi]/angiotensin receptor blocker [ARB] use) were included as independent variables. Principal component analysis (PCA) was performed to correct for multiple comparisons using biomarker measurements from AHF patients with and without diabetes as categorical variables.^{11,12,13} The use of PCA is often used in -omics based studies, where there is a natural correlation between markers reflective of the similar underlying pathophysiological processes¹⁸ and PCA based correction for multiple comparisons has been suggested to be more effective than Bonferroni correction^{18,19}. PCA has been previously successfully used in correcting for multiple comparisons in pairwise correlations in other disease states.¹¹ A total of 37 principle components cumulatively explained $> 95\%$ of the variation observed in the dataset when comparing both groups. The corrected significance level for multiple testing was thus set at $P < 0.05/37$, equating to an adjusted p-value cut-off of

0.001351. Next, a Spearman's rank correlation coefficient was calculated for each possible biomarker pair in the cohort of patients with diabetes and the procedure was repeated for patients without diabetes. This resulted in two sets of R values with associated p-values for both groups. To adjust for multiple testing, only those correlations passing the adjusted p-value cut-off calculated from the PCA were deemed statistically significant and subsequently retained. These significant correlation coefficients were then graphically displayed as heatmaps to reveal how biomarkers within patients with diabetes and non-diabetes clustered together. Network analyses were then performed to analyze the cumulative associations between biomarkers in patients with and without DM. To better position the global associations of these biomarker interactions, biomarker data together with statistical data pertaining to each group of patients were utilized in a network analysis. Results of network analysis derived from patients with and without diabetes are shown in figures 2 and 3 respectively. The network analysis graphically represents two major findings: (1) whether biomarkers are correlated (i.e. how closely the levels of two biomarker levels rise and fall). The strength of each biomarker-biomarker correlations is graphically represented by the thickness of the line connecting the biomarkers; (2) how biomarkers correlate (i.e. how closely the levels of biomarkers correlate with multiple neighboring biomarkers). How closely a biomarker clusters with its neighbor is graphically represented by the size of the circle (or hub). We also assessed the correlation between individual biomarkers and routine clinical variables including height, weight, blood pressure, respiratory rate, pulse, and LVEF. The correlation coefficients were plotted as a correlogram.

Statistical analyses were performed using the STATA (version 11.0, STATA Corp, College Station, TX, USA), R (version 2.15.1, R Foundation for Statistical Computing, Vienna, Austria) software, and SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Cor).

Results

Baseline demographic and clinical characteristics

Compared to patients without diabetes, those with diabetes had higher prevalence of ischemic heart disease, previous coronary intervention, renal dysfunction, body mass index, and other traditional coronary risk factors (table 1).

Biomarker expression

Patients with diabetes had consistently higher levels of inflammatory biomarkers compared to patients without DM, which included PIGR, RAGE, TNFR1A, GDF-15, WAP4c (figure 1, table 2). The remodeling specific biomarkers (syndecan-1 and GAL-3), atherosclerosis markers (LTBR), lipids (triglycerides, total cholesterol) and renal markers (creatinine, BUN, NGAL, KIM1) were significantly higher in patients with diabetes compared to those without diabetes. The remodeling marker periostin, cardiomyocyte stretch marker BNP and thrombosis marker D-Dimer were higher in patients without diabetes compared to patients with diabetes. After adjustment for age, sex, clinical covariates, and ACEi/ARB use, pentraxin-3, TNFR-1a, D-dimer, periostin, BNP, VEGFR, angiogenin, LTBR, NGAL, and KIM-1 were significantly different between patients with and without diabetes (adjusted p-value <0.05; supplementary appendix table 2).

Biomarker correlation with network analysis

Statistically significant biomarker correlations that survived multiple testing within the diabetes and non-diabetes groups are depicted as heatmaps (supplementary appendix figures 1 and 2 respectively, and supplementary appendix table 3 and 4 respectively). The strongest inter-biomarker correlation observed in patients with diabetes involved the association between glucose and triglycerides (figure 2, supplementary appendix table 3). Furthermore, a renal-potassium-glucose correlation emerged among patients with diabetes; potassium was

strongly correlated with glucose, creatinine, and NGAL (figure 2). Periostin had the largest hub, suggesting that this biomarker strongly clusters around the neighboring inflammatory biomarkers CRP and IL6. Among patients without diabetes, these associations were not seen (figure 3); however, BUN is strongly correlated with troponin and BNP associates with AST. Furthermore, among patients without diabetes, the angiogenesis marker angiogenin appeared to strongly cluster with BUN and another angiogenesis marker (TROY). Periostin does not appear to significantly associate or cluster with other biomarkers among patients without diabetes.

Among the association between biomarkers and clinical variables, the most significant correlations occurred with LVEF and the biomarkers. Among patients with diabetes, LVEF was strongly associated with d-dimer, RAGE, and IL6. Among patients without diabetes, the strongest clinical and biomarkers correlations were seen with LVEF and d-dimer, NT-proCNP, and BNP (supplementary figure 3 and 4).

Discussion

To our knowledge this is the first network analysis to identify correlations and clusters of associations using an extensive panel of biomarkers in patients with and without diabetes in AHF. The two main findings of the present study were: (1) There are significant differences in biomarker levels across a domain of pathophysiologic processes among patient with and without diabetes in AHF; (2) cardiac remodeling-fibrosis-inflammation biomarkers strongly cluster closely among patients with DM - a finding not seen among patients without diabetes; (3) a renal-potassium-glucose correlation of potassium, glucose, creatinine, NGAL, and galectin-3 was seen among patients with diabetes. These results suggest that in the setting of AHF, distinct pathophysiologic processes are likely to be present among patients with

diabetes compared to those without DM.

Differences in biomarker profiles

The association of diabetes, renal disease, inflammation in humans has been described.^{6,7} Our results expand on these findings by demonstrating that even after multivariable adjustment the inflammatory marker TNF-R1a and renal markers (KIM1, NGAL) were significantly higher in patients with diabetes compared to those without diabetes. Periostin was significantly lower in patients with diabetes even after multivariable adjustment. Periostin knockout models are associated with increased cardiac and valvular fibrosis.²⁰ Treatment with periostin in cardiac infarction models results in improvement in cardiac contractility and ventricular remodelling.²¹ Lower periostin level may suggest a reduced ability to undergo adaptive ventricular remodelling among patients with diabetes compared to patients without diabetes. In prior rat studies of diabetic cardiomyopathy, myocardial expression of periostin was higher.²² The finding of lower periostin in our study may reflect differences in location of periostin measurement (serum versus myocardium) or clinical state (acute versus chronic HF). Overall, the significant differences in biomarker levels across a range of pathophysiologic pathways provides empiric clues that different disease mechanisms may be present in patients with and without diabetes and AHF.

Our analysis revealed a higher BNP among patients without diabetes even after multivariable adjustment. While some reports have suggested a higher BNP associated with diabetes²³, other large population studies and clinical trials have demonstrated lower BNP levels among patients with diabetes and HF^{3,4}. The lower BNP seen among patients with diabetes and HF likely reflects the higher incidence of obesity, which is known to associate with lower natriuretic peptide levels²⁴.

Correlation and clustering of biomarkers

Another important finding of the present study was that periostin clustered around established markers of fibrosis and inflammation (CRP and IL6) in patients with diabetes. Our results extend upon work from existing experimental models. In diabetic rats, compared to controls, periostin is closely associated with ventricular fibrosis and adverse cardiac remodeling.²² The use of valsartan in diabetic rats significantly improved ventricular remodelling and markers of fibrosis by possibly targeting the periostin-pathway²². Our findings suggest that in patients with AHF, the mechanisms of cardiac remodelling, fibrosis, and inflammation, are closely related; these findings were not observed in patients without diabetes. Such analyses of blood biomarkers have been used to examine the pathophysiologic mechanisms in patients with AHF¹² and among patients HF with reduced and preserved ejection fraction.¹³ IL-6 and CRP levels are similar among patients with and without diabetes reflecting the acute inflammation seen in AHF. However, in patients with diabetes, periostin associates with inflammatory proteins IL-6 and CRP; while further evaluation is needed, these results may reflect a possible mechanistic link between periostin and acute inflammation. Periostin did not cluster around other inflammatory markers that were elevated among patients with diabetes including RAGE and GDF-15. While all of these biomarkers act through the inflammatory cascade in some capacity, their different mechanisms of action are likely reflected in the lack of clustering seen between these proteins^{7,21}. The role of the periostin-pathway and inflammation among patients with diabetes and HF warrants further evaluation.^{21,25}

In addition to the periostin clustering, a renal-potassium-glucose correlation was present among patients with diabetes. These result suggests that potassium levels among patients

with diabetes are more sensitive to changes in renal function. One potential explanation may relate to baseline medications; however, patients with diabetes were only slightly more likely to be on ACE-i/ARB compared to patients without diabetes (78% vs. 74%; $p=0.03$; table 1) and were equally likely to be on mineralocorticoid receptor antagonists (42% vs. 46%; $p=0.1$; table 1). A history of diabetes has been shown to be an independent predictor of hyperkalemia among patients with and without HF²⁶. Our results extend on prior analyses suggesting that among patients with diabetes, potassium and glucose levels are correlated through insulin mediated regulation, and this correlation is significantly influenced by insulin-resistance and kidney disease²⁷. Our findings have clinical implications; hyperkalemia among patients with AHF may portend a worse prognosis,²⁶ clinicians need to aggressively monitor renal function and optimize potassium levels among patients with diabetes.

Among patients without diabetes, a cardiac stretch-hepatic relationship was seen as BNP correlated strongly with AST. Abnormal liver enzymes in patients admitted with AHF are correlated with worse prognosis²⁸; our results suggest that changes in liver enzymes among patients with diabetes may be more sensitive to volume status and changes in cardiomyocyte stretch as reflected by BNP.

The network analysis results were further supported by the association of biomarkers and clinical data. The inflammatory molecules RAGE and IL6 were the most strongly correlated with LVEF in patients with diabetes while NT-proCNP and BNP were the most strongly correlated with LVEF in patients without diabetes. These results suggest that inflammation and oxidation may be a dominant contributor to myocardial function among diabetics in AHF, while cardiomyocyte stretch may be a major contributor to myocardial function among patients without diabetes.

Clinical implications and future direction

Our results suggest that among patients with and without diabetes in AHF different underlying mechanisms of disease may exist. Periostin may be playing a central role in the pathogenesis of HF among patients with DM, and therapies targeting the periostin-pathway may represent a novel treatment strategy.^{21,22,25} Therapies that modulate the periostin-pathway are being explored in cardiac models of heart failure and myocardial infarction.²⁹ Valsartan has demonstrated improvements in ventricular remodeling in diabetic rats potentially through the periostin-pathway.²² It is unclear whether angiotensin converting enzyme inhibitors (ACEi) targets the periostin-pathway. While there are no direct head to head comparison of ACEi and angiotension receptor blockers (ARB) in patients with diabetes and HF, comparative analyses have suggested that ARBs may be superior than ACEi in the setting of diabetic nephropathy.³⁰ Studies evaluating strategies that target the periostin-pathway, with ARBs or other therapies, may represent a future direction of research among patients with diabetes and HF. In addition to cardiac disease, periostin plays a significant role in the mineralization of bone extracellular matrix³¹ which has significant implications for osteoporosis development among patients with diabetes³². Further evaluation of Bone Morphogenetic Protein -1 (BMP-1) and lysyl oxidase (LOX) activity, which are associated with periostin function, would be required to clarify the role of the periostin-pathway in patients with diabetes and HF.³¹ Furthermore, given the renal-potassium-glucose correlation seen in our analysis, strategies to optimize potassium may represent a strategy to improve outcomes among patients with diabetes and will need to be evaluated in prospective studies.

Strengths and Limitations

This study is affected by the limitations of post hoc analyses, necessitating cautious interpretation. PROTECT had no specific design to warrant sufficient power for analyses of

the diabetic subgroup. Information on anti-inflammatory medications was also not available. In addition, while patient medications may potentially affect biomarker levels, we did not adjust for this given the more descriptive nature of this analysis. In our study, we have used network analysis as a way to determine the underlying pathophysiologic mechanisms and this method has been utilized in a variety of other analysis.^{11,12,13} While co-morbidities and medications differed among patients with and without diabetes, these were not adjusted for in the clustering analysis as the intent of our analysis was to reflect the overall subgroups of patients with and without diabetes. Type of diabetes (i.e. type 1 vs. type 2), degree of glycemic control (through HbA1c), or duration of diabetes may potentially influence our results but were not collected in the PROTECT trial. Our findings are predominantly hypothesis generating; however, the conservative p-values used in our principle component analysis ensures a statistically more robust result.

Conclusion

Using network analyses, among AHF patients with and without diabetes, our findings suggest that cardiac remodeling, inflammation, and fibrosis - as reflected by the clustering of periostin, CRP, and IL6 - are closely associated among patients with diabetes. Furthermore, renal function, potassium levels, and glucose are closely correlated among patients with diabetes. These findings were not seen in patients without diabetes. Our study suggests that different pathophysiology pathways may be active among patients with and without DM and AHF. Further research will be needed to explore these results.

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measurements

Conflicts of interest

Abhinav Sharma is supported by the Alberta Innovates Health Solution Clinician Scientist Fellowship. He has received research support from Roche and the Canadian Cardiovascular Society Bayer Vascular Research grant. Beth Davison and Gad Cotter are employees of Momentum Research Inc., which has provided consulting services to NovaCardia, Merck, Corthera, Novartis, Singulex, ChanRx, Laguna Pharmaceuticals, Sorbent Therapeutics, Celyad SA, Trevena, Amgen, and Anexon. Marco Metra has received honoraria and reimbursements from NovaCardia, sponsors of the study, and from Merck, which purchased the rights to rolofylline after completion of the PROTECT pilot study. Daniel Bloomfield is an employee of Merck & Co. John Cleland reports grants and personal fees from MSD, while conducting the study, grants and personal fees from Amgen, grants and personal fees from Novartis, personal fees from Stealth Biopharmaceuticals, personal fees from Servier, grants and personal fees from Bayer, and personal fees from Sorin, outside the submitted work. Howard Dittrich was an employee of NovaCardia and a consultant to Merck. Michael Givertz has received institutional research support and served on a scientific Advisory Board for Merck. Piotr Ponikowski has received honoraria from Merck, consulting fees from Vifor Pharma and Amgen, Inc., honoraria from Vifor Pharma, and travel/accommodation expenses covered by Vifor Pharma and Amgen, Inc. John Teerlink has received research funds and consulting fees from Merck, the makers of rolofylline for conducting this study and has also received research funds and/or consulting fees from Amgen, Cytokinetics, Novartis, Relypsa, Trevena, and ZS Pharma for research in related areas. Adriaan Voors has received speaker/consultancy/research fees from AstraZeneca, Bayer, BMS, Boehringer, Cardio3Biosciences, GSK, Merck/MSD, Novartis, Servier, Spingotec, Stealth, Trevena, Vifor.

All other authors reported that they have no conflict of interest to declare.

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Figure Legends:

Figure 1: Percentage difference in baseline biomarker levels between patients with and without diabetes mellitus admitted with acute heart failure.

% change calculated by subtracting the median value of the baseline biomarker in patients with diabetes mellitus from the median value of the baseline biomarker in patients without diabetes mellitus and dividing the results by the median value of the biomarker with diabetics. *denotes statistically significant levels between diabetic and non-diabetic levels ($P < 0.05$)

Abbreviations: ALT alanine aminotransferase; AST aspartate aminotransferase; BUN blood urea nitrogen; BNP: B-type Natriuretic Peptide; CRP C-reactive protein; GDF-15 growth differentiation factor 15; PCT procalcitonin; PIGR Polymeric immunoglobulin receptor; PSAP-B Prosaposin B; RAGE Receptor for advanced glycation end product; RBC red blood cell; WAP4C WAP Four-Disulphide Core Domain Protein HE4; WBC white blood cells; TNF-R1a tumor necrosis factor alpha receptor 1; ST2 Soluble ST2; VEGFR vascular endothelial growth receptor; proADM pro-adrenomedullin; NT-proCN: N-terminal pro-C-type natriuretic peptide; LTBR lymphotoxin beta receptor; ESAM endothelial cell-selective adhesion molecule; NGAL neutrophil Gelatinase-associated Lipocalin; ET1 endothelin-1; IL6 interleukin-6; KIM1 kidney injury molecule 1.

Figure 2: Network analysis between biomarkers in patients with acute heart failure and diabetes mellitus

Biomarkers are represented as circular hubs, with associations depicted as connecting lines. The thickness of the line is directly proportional to the strength of the correlation and the size of the hub reflects the clustering coefficient (additive correlations of neighboring pairs of biomarker associations). The color of the circular hubs (biomarkers) and lines (associations) represent the strength of the clustering coefficient and strength of inter-biomarker correlations respectively; these range from blue; strongest, to orange; weakest. Of all statistically significant associations depicted, glucose and triglycerides are the most strongly correlated with each other, reflected by the thickness of the line. Periostin has the largest hub, reflecting strong additive correlations with CRP and IL6.

Figure 3: Network analysis between biomarkers in patients with acute heart failure and without diabetes mellitus

Biomarkers are represented as circular hubs, with associations depicted as connecting lines. The thickness of the line is directly proportional to the strength of the correlation and the size of the hub reflects the clustering coefficient (additive correlations of neighboring pairs of biomarker associations). The color of the circular hubs (biomarkers) and lines (associations) represent the strength of the clustering coefficient and strength of inter-biomarker correlations respectively; these range from blue; strongest, to orange; weakest. While no one dominant association was seen, the strongest correlation appears to be with AST and BNP. Angiogenin has the largest hub, reflecting strong additive correlation with BUN and TROY.”