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Original Article

A comparative analysis of novel cardiovascular biomarkers in patients with chronic heart failure

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ABSTRACT

Background: Heart failure (HF) with reduced ejection fraction remains a major therapeutic challenge. The aim of this study was to investigate the role of novel cardiovascular biomarkers, i.e. soluble suppression of tumorigenicity (sST2), growth-differentiation factor-15 (GDF-15), soluble urokinase plasminogen activator receptor (suPAR) and heart-type fatty acid binding protein (H-FABP) in patients with ischaemic (ICM) or dilative cardiomyopathy (DCM).

Materials and methods: A total of 200 patients were enrolled in this study: 65 were diagnosed with DCM and 59 patients suffering from ICM were included. 76 patients without coronary artery disease or signs of heart failure were included as controls. Plasma samples of all patients were analyzed by use of ELISA.

Results: Levels of sST2, suPAR and H-FABP were significantly higher in ICM and DCM patients compared to the control group ($p < 0.0001$). However, there were no significant differences between ICM and DCM in biomarker levels. Ejection fraction correlated inversely with cardiac biomarkers (sST2 $p < 0.0001$, GDF-15 $p = 0.0394$, suPAR $p = 0.0029$, H-FABP $p < 0.0001$). Similarly, CRP levels also showed a positive correlation with cardiac biomarkers. Renal insufficiency ($p < 0.0001$) and diabetes (sST2 $p = 0.0021$, GDF-15 $p = 0.0055$, suPAR $p = 0.0339$, H-FABP $p = 0.0010$) were significantly associated with a rise in cardiac biomarkers.

Conclusion: Novel cardiovascular biomarkers such as ST2, GDF-15, uPAR and H-FABP could offer a great potential for more precise diagnostic in ICM and DCM patients. H-FABP was the most promising marker in our study, followed by sST2, uPAR and GDF-15. Additional prospective studies will be necessary to further evaluate the potential clinical benefits in routine treatment of HF.

1. Introduction

Heart failure with reduced ejection fraction affects over 20 million people worldwide with an overall prevalence of 2% in adults [23]. With a prevalence increasing exponentially up to 10% in people over 70 years of age, heart failure represents one of the leading causes of mortality and morbidity especially in elderly patients [3,20]. As a consequence, it contributes to a rising number of hospital admissions with an overall admission rate of 1%–2% per year, again increasing with age [23,31]. In patients aged over 65, heart failure even constitutes the number one reason for hospital admission [3,23,31].

With costs estimated over a hundred billion dollars per annum, heart failure remains a major economic factor, with a great potential to reduce health care costs by optimizing early diagnosis and therapy [7,23]. While diagnosis and treatment regimens of heart failure are made relatively easy by widely established algorithms based on parameters such as clinical symptoms, biomarkers and echocardiography as well as coronary angiography, detailed pathogenesis and mechanisms of heart failure still remains subject of extensive investigation [23].

Ischaemic cardiomyopathy (ICM) represents the most common of all entities in heart failure patients [5,12]. Despite significantly improved strategies for reperfusion and management of coronary artery disease, ischaemic heart disease remains the single leading cause for heart failure with reduced ejection fraction [5,12]. However, with coronary artery disease still being the most influential factor, a normal coronary angiogram does not rule out ischaemic cardiomyopathy, as impaired microcirculation or inflammation processes can result in

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permanent myocardial damage as well [23]. Besides ICM, dilated cardiomyopathy (DCM) represents a second highly important entity in heart failure patients [13,23]. The pathogenesis resulting in DCM is considered to be a combination of numerous genetic and environmental influences, mainly due to inflammation, resulting in dilatation and impaired ventricular function [13,22].

A broad spectrum of novel cardiovascular biomarkers is currently under investigation. One of them, soluble suppression of tumorigenicity (sST2) was first described in 1989 as a member of the interleukin-1 (IL-1) receptor family [6,19]. To our current knowledge, ST2 can act as a membrane bound receptor (ST2L) but also as a secreted protein (soluble ST2; sST2) [15,19]. However, it took until 2005 to identify Interleukin-33 (IL-33) as the functional ligand for the ST2L receptor [15,19,28]. The signaling axis of IL-33 and ST2 was first described in the pathophysiological setting of inflammation and immunity [15,19,28]. IL-33 secretion is triggered by local tissue inflammation and also by necrotic cell death as a danger signal [27,28]. By acting as kind of a decoy receptor for IL-33 via binding to IL-33, sST2 inhibits IL-33/ST2L signaling [6,27,35]. This supported the hypothesis that sST2 possesses anti-inflammatory and immunosuppressive features [10,15,19]. In the early 2000s, haemodynamic stress and cardiomyocyte strain was described as a second major triggering factor leading to sST2 release [6,19].

Growth-differentiation factor-15 (GDF-15) is a member of the transforming growth factor β-family and has also been described as a stress-responsive biomarker of cardiac and vascular disease [1,24]. By oxidative stress and inflammation, GDF-15 expression is up-regulated [1,17]. It has been introduced as a prognostic marker for patients with coronary artery disease (CAD) especially in the setting of acute non-ST-elevation myocardial infarction (NSTEMI) [8,16].

Soluble urokinase plasminogen activator receptor (suPAR) is a pro-inflammatory marker and was shown to be associated with systemic inflammatory response syndrome, malignancies and cardiovascular disease [29,30,32]. Furthermore, suPAR has been shown to correlate with the risk of atherosclerosis [29]. Moreover, researchers from Denmark provided evidence that suPAR levels correlated with cardiovascular disease, type 2 DM, cancer, and mortality [9].

Heart-type fatty acid binding protein (H-FABP) is a low molecular weight protein (14-15 kDa) that is expressed in myocardial cells [26]. Like troponin, H-FABP can be detected in the blood of patients with myocardial ischaemia once cardiomyocyte damage occurs [14,25]. The primary results of previous studies suggest that H-FABP can indicate very early ischaemic damages in human myocardium [14,25]. Niizeki et al. have shown that elevated H-FABP levels at hospital admission were associated with increased risk of deaths and non-fatal cardiac adverse events even in troponin-negative patients [21].

With the aim to better understand the molecular mechanisms in heart-failure patients, we sought to perform a head-to-head analysis of these four novel cardiovascular biomarkers in patients suffering from ICM or DCM.

2. Methods

2.1. Study population

A total of 200 non-consecutive patients admitted to the University Hospital Jena in Germany were enrolled in this retrospective single-center study. 65 patients that were diagnosed with DCM and 59 patients that suffered from ICM were included. 76 patients without coronary artery disease or signs of heart failure that underwent coronary angiography due to angina pectoris were included as control patients. Diagnosis of ICM was made according to the guidelines issued by the European Society of Cardiology (ESC). Invasive testing with coronary angiography was performed after initial evaluation and clinical examination. Moreover, all patients underwent assessment of medical history, laboratory parameter analysis and transthoracic echocardiography. In contrast, diagnosis of DCM was defined according to ESC guidelines when dilatation of the left ventricle was present together with signs of heart failure but without evidence of any relevant coronary artery disease. Invasive assessment by coronary angiography was also performed in all DCM and control patients to exclude CAD and other causes of myocardial dysfunction.

All patients in the ICM and DCM groups suffered from heart failure with reduction in left ventricular ejection fraction and were screened during out-patients visits at our specialist clinic for patients suffering from HF. All patients were recruited and examined in a non-decompensated state by an experienced physician in the treatment of HF. Patients showing clinical signs of acute HF were not enrolled in this study.

Blood specimens for serum sample analysis were obtained from all study patients after informed consent. Serum samples were stored at −80° until ELISA measurements were performed.

Exclusion criteria were: active infection, malignant disease or advanced stages of renal failure (as indicated by a glomerular filtration rate < 30 ml/min). The study was approved by the local ethics committee at the University Clinic Jena and was conducted in accordance with the Universal Declaration of Helsinki.

2.2. Laboratory analysis

Analysis of standard clinical laboratory parameters was performed at the Department of Clinical Chemistry (University Hospital Jena). These analyses included high-density lipoprotein (HDL; mmol/l), low-density lipoprotein (LDL; mmol/l), triglycerides (mmol/l), and C-reactive protein (CRP, mg/l) as well as hematological parameters. Glomerular filtration rate was calculated according to the CKD-EPI equation. [2].

Serum levels of sST2, GDF-15, suPAR and H-FABP were determined by commercially available ELISA kits (DuoSet ELISA, DY523B, DY957, DY807 and DY1678, R&D Systems, USA). ELISA assays were performed in accordance with instructions supplied by the manufacturer. In short, serum samples and standard proteins were added to the multi-well plate coated with the respective capture antibody and incubated for 2 hours. Plates were then washed using washing buffer (Tween 20, Sigma Aldrich, USA and phosphate buffered saline solution). In the next step, a biotin-labelled antibody was added to each well and incubated for another 2 hours. ELISA plates were washed another time and a streptavidin-horseradish-peroxidase solution was added. After adding tetramethylbenzidine (TMB; Sigma Aldrich, USA), a colour reaction was achieved. Optical density was measured at 450 nm on an ELISA plate-reader (iMark Microplate Absorbance Reader, Bio-Rad Laboratories, Austria).

We also sought to investigate IL-33 serum levels in comparison to qith sST2, however, no differences between the groups were found (data not shown).

2.3. Statistical analysis

Statistical analysis was performed using GraphPad-Prism software (GraphPad-Software, La Jolla, CA, USA) and SPSS (22.0, SPSS Inc., USA). The Kolmogorov-Smirnov test was used to assess normal distribution of parameters in the study population. Normally distributed parameters were given as mean + standard error of the mean. Means were compared by student's *t*-test. As demographic parameters and biomarker concentrations were not normally distributed, all values are given as median and inter-quartile range. Median values were compared using the Mann-Whitney-*U* test. Correlation analysis was performed using Spearman's rank-correlation coefficient. ROC analysis was performed and an optimal cut-off was calculated by means of the Youden Index. AUCs were compared as described by Hanley and McNeil [11]. Differences between NYHA stages were compared using the Kruskal-Wallis test with Dunn's post-test for multiple comparisons. A $p < 0.05$ was considered to be statistically significant.

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