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ORIGINAL ARTICLE

Soluble Glycoprotein 130 and Heat Shock Protein 27 as Novel Candidate Biomarkers of Chronic Heart Failure with Preserved Ejection Fraction

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Background Despite their importance, the current clinical biomarkers of chronic heart failure have limitations. In this study, soluble glycoprotein 130 (sgp130), heat shock protein 27 (hsp27), dipeptidyl peptidase IV (dpp4) and cathepsin S (CTSS) were tested for their potential as novel biomarkers for diagnosing chronic heart failure (CHF) with preserved ejection fraction.

Methods We compared the circulating levels of sgp130, hsp27, dpp4, and cathepsin S in patients with CHF with preserved ejection fraction (n=50) and in controls (n=50), determined how well these candidate biomarkers distinguish patients with CHF from controls, and assessed whether these candidates are superior to N-terminal pro brain natriuretic peptide (NT-pro-BNP) as diagnostic tools.

Results After adjusting for clinical covariates, patients with CHF showed significantly higher mean concentrations of sgp130 (317.38pg/ml vs. 215.90 pg/ml), hsp27 (2601.02 pg/ml vs. 923.61 pg/ml) and NT-pro-BNP (982.35 pg/ml vs. 331.99 pg/ml), but not dpp4 (6930.9 4pg/ml vs. 7081.37 pg/ml) or CTSS (1050.46 pg/ml vs. 984.96 pg/ml), than did controls. In the receiver operating characteristic curve analysis, hsp27 showed the most notable difference between CHF patients and controls, with the largest area under the curve (AUC) (0.920); the AUC values for sgp130 and NT-pro-BNP were 0.877 and 0.882, respectively.

Conclusions Soluble glycoprotein 130 and hsp27 are novel candidate biomarkers for diagnosing CHF with preserved ejection fraction and thus warrant further investigation; neither dpp4 nor CTSS showed promise as biomarkers of CHF.

Keywords Chronic heart failure • Biomarkers • Soluble glycoprotein 130 • Heat shock protein 27 • Dipeptidyl peptidase IV • Cathepsin S

Introduction

Chronic heart failure (CHF), a major and growing public health problem, is a consequence of cardiac overload and injury and is characterised by clinical symptoms related to cardiac impairment. Clinical biomarkers, such as brain natriuretic peptide (BNP) and its precursor, N-terminal pro brain natriuretic peptide (NT-pro-BNP), play important roles in

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evaluating chronic heart failure [1]. However, natriuretic peptides can be secreted into circulation by cardiomyocytes under stress conditions, including not only heart failure, but also myocardial ischaemia, inflammation and trauma. Concentration of circulating natriuretic peptides is strongly influenced by age, sex, body size, and renal function. Therefore, neither BNP nor NT-pro-BNP has satisfactory specificity and positive predictive value in diagnosing CHF [2]. Complementary novel biomarkers are in urgent need for clinical practice. Despite great enthusiasm directed to the search for new biomarkers, few new protein markers have been approved by the FDA for the clinical diagnosis of heart failure.

Proteomics technologies have recently emerged as powerful tools for identifying new biomarkers and pathways without a previously known association with heart failure [3]. Pericardial fluid (PF) is proximal to the heart and can be thought of as a biochemical 'window' onto the heart. This fluid contains a high concentration of a large repertoire of proteins that are shed and secreted by the heart and is therefore considered a promising source of new biomarkers of heart diseases [3,4]. Thus, we searched for candidate CHF biomarkers in PF using a proteomics strategy and validated these markers in blood samples.

We previously established the first proteome database for normal human PF, which includes 1007 nonredundant proteins. According to the bioinformatics analysis, the majority of PF proteins appeared to be released from the heart; additionally, a series of recognised biomarkers of various types of CVD are present in the PF proteome [5].

In this study, we utilised bioinformatics analyses to investigate the potential involvement of heart-derived PF proteins in CHF. This analysis yielded four proteins (soluble glycoprotein 130 (sgp130), heat shock protein 27 (hsp27), dipeptidyl peptidase IV (dpp4) and cathepsin S (CTSS)) as new biomarker candidates for clinical validation in blood samples. We speculated that these four candidates may be present at significantly different levels in CHF than in controls and may be novel biomarkers complementary to BNP and NT-pro-BNP, because they reflect distinct pathological mechanisms. Accordingly, we (i) compared the circulating levels of sgp130, hsp27, dpp4, cathepsin S and NT-pro-BNP in people with CHF and controls without HF, (ii) determined the potential of these candidates to distinguish between people with CHF and controls, and (iii) compared the diagnostic ability of these candidates with that of NT-pro-BNP.

Methods

Selection of Candidate Biomarkers

In a previous study [5], a total of 674 heart-derived proteins were identified in the human PF proteome, and 190 of these heart-derived proteins seemed to be localised in the extracellular region and be secreted into circulation. To determine whether there was an association with CHF, in this study, we performed a bioinformatics analysis using Pathway Studio 8.0 (Ariadne Genomics, Rockville, MD, USA) and the ResNet Mammalian Database (detailed information about the potential involvement of PF proteins in heart failure can be found in *Supporting information data file 1*). A total of 57 proteins were found to potentially be involved in CHF (Figure 1). Of these proteins, 21 had previously been recognised as CHF biomarkers, and seven had been reported to be unsuitable as CHF biomarkers. After searching for the availability of corresponding ELISA kits, four novel biomarker candidates were chosen and validated: soluble glycoprotein 130 (sgp130), heat shock protein 27 (hsp27), dipeptidyl peptidase 4 (dpp4) and cathepsin S (CTSS).

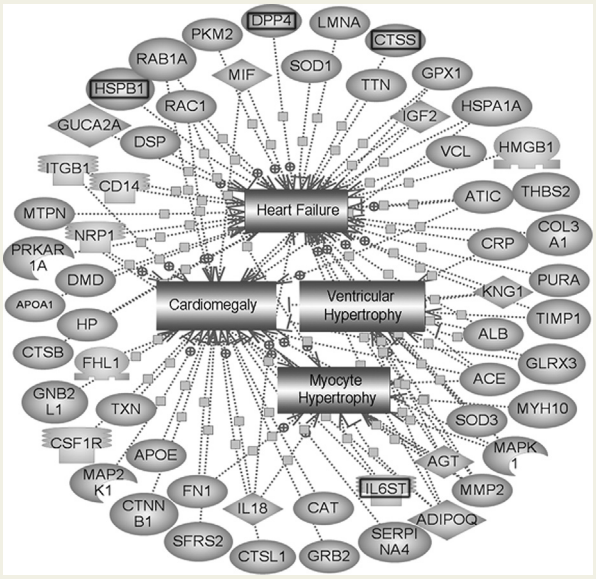

Figure 1 Proteins related to heart failure by bioinformatics analysis. Proteins in rectangle were finally selected as follows: DPP4 (dipeptidyl peptidase 4), CTSS (cathepsin S), HSPB1 (heat shock protein 27, hsp27) and IL6ST (soluble glycoprotein 130, sgp130).

Study Population

The study conforms to the principles outlined in the Declaration of Helsinki. The trial was also approved by the ethics committee of Nanjing First Hospital affiliated with Nanjing Medical University.

The diagnosis of CHF was made according to the Framingham criteria [6]. Chronic heart failure patients who had experienced symptoms or signs of heart failure in the month before the study began were recruited at Nanjing First Hospital. The diagnosis of CHF was clinically confirmed in each instance by a trained physician. Chronic heart failure was identified as the presence of characteristic symptoms and signs regardless of natriuretic peptide levels. Blood samples were collected after in-hospital stabilisation to ensure that all patients were uniformly sampled when in a clinical status of compensated CHF. According to their symptoms and signs, all of these patients were classified as stage C CHF.

The controls included participants without heart disease as confirmed by their history, clinical tests and heart tests. This group was randomly recruited from healthy adults at the