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Heat shock protein 27 acts as a predictor of prognosis in chronic heart failure patients

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ABSTRACT

Background: Heat shock proteins (HSPs) represent intracellular mechanisms of stress response. Clinical implications of their (systemic) expression in patients with chronic heart failure (HF) remain inconclusive.
Methods: In outpatients with chronic stable HF plasma HSP27 levels were measured using ELISA. Patients were followed for a minimum of one year, and a multivariate Cox proportional hazard model was built for cardiovascular death or HF-associated hospitalisations.
Results: A total of 134 patients with chronic HF (mean age 71 ± 10 years, 34% female, mean LVEF 36 ± 12%) were included. During a mean follow-up of 527 ± 260 days, 44 patients (33%) experienced an event. Mean time to event was 350 ± 236 days. In a Kaplan-Meier survival analysis HSP27 levels above the median (3820 pg/ml) indicate a higher risk for an event ($p = 0.03$). Increased HSP27 levels remained an independent predictor of events (HR, 2.33 CI 95% 1.12–4.87, $p = 0.024$) even after adjustment for age, gender, NT-proBNP, LVEF, aetiology, smoking status, kidney function and NYHA class.
Conclusions: HSP27 is an independent predictor of prognosis in chronic HF. Our findings suggest that HSP27 may improve risk-stratification in chronic HF beyond known prognostic predictors.

1. Introduction

Chronic heart failure (HF) is a major health problem affecting about 2% of the overall industrialised population, exceeding 10% in the elderly population (> 70 years) and rising in prevalence each year [1,2]. Chronic HF is characterised by ongoing structural or functional myocardial impairment leading to insufficient cardiac output that does not meet the body's demands. Chronic HF comprises both patients with preserved left ventricular ejection fraction (LVEF) (HFpEF) and patients with reduced LVEF (HFrEF), recently another patient's subset has been introduced: patients with mid-range LVEF (HFmrEF) [2,3]. Worldwide, mortality in chronic HF patients after admission is 17–45% within 1 year and the majority of patients dies within 5 years [4]. Hospitalisations also contribute significantly to poor prognosis as up to 40% of all patients are rehospitalised within one year after admission for chronic HF [5,6]. Due to the excessive burden of chronic HF an accurate model to predict prognosis in chronic HF patients is needed to adapt the present therapy regime in single patients [7]. Current predictors of chronic HF focus on specific populations of HF patients and most models provide only moderate accuracy [7,8]. A variety of variables (e.g. age, sex, NYHA functional class, exercise capacity, ejection fraction and comorbidities) have been associated with mortality and rehospitalisation in chronic HF [7,9]. Complimentary to that, systemic biomarkers such as brain natriuretic peptide (BNP) and N-terminal pro brain natriuretic peptide (NT-proBNP) serve as important tools in the evaluation and prognostic risk assessment of chronic HF [7,8]. Nowadays chronic HF is considered a complex syndrome, which is associated with chronic low-grade inflammation beyond other systemic pathologies [10]. This sets stage for other laboratory biomarkers that can be used to assess concomitant pathophysiological mechanisms in HF.

Abbreviations: AKT, Protein kinase B; BNP, brain natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HF, heart failure; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduce ejection fraction; HSP, heat shock protein; IFN-γ, interferon γ; IL-1β, interleukin 1β; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; PI3K, Phosphatidylinositol-4,5-bisphosphate 3-kinase; TLR2, Toll-like receptor 2; TNF-α, tumour necrosis factor α

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Constant low-grade inflammation in HF has been addressed by experimental therapies [11]. Even though mechanisms of inflammation in cardiac tissue repair are well understood, the role of inflammation in chronic HF is still unknown [10]. It is already well known that chronic HF induces circulating inflammatory cytokines (e.g. TNF-α, IFN-γ, IL-1β, IL-6, IL-17 and IL-18) and some of those also provide prognostic value [10,12,13].

In this context, the role of heat shock proteins (HSPs) is insufficiently investigated. The HSP family represents highly conserved intracellular ATP-independent proteins with a chaperone function [14–16]. They are characterised by a conserved α-crystallin domain that is located between a variable C- and N-terminal [15]. HSPs fulfil their function as chaperones by stabilizing early unfolding proteins and in consequence prevent protein aggregation. However, they cannot rescue already unfolded proteins. They do so constitutively but expression of most HSPs rises under pathophysiological stressors such as ischemia, free radicals or heat, which may be one of the oldest regulatory mechanisms. A preferred spectrum of substrates for HSPs has not been identified [14,15,17,18]. HSPs are present in different forms (mono-, di- and oligomers) [19]. Their activity is highly dependent on its organisation: as oligomers they are in an “inactive state” and transform to an “active state” as they dissociate to dimers as a consequence of stress stimuli [17,19]. Phosphorylation of HSPs as a response to stress is known to induce dissociation of oligomers and to activate HSPs [17]. HSPs are involved in a plethora of molecular processes such as cellular growth and differentiation, interaction with cytoskeleton and apoptosis [15].

As response to intracellular stress they are released from cells to interact with surrounding and distant cells by entering the blood stream [14]. As HSPs are present in a variety of tissues they are associated with a myriad of diseases. However, it is difficult to determine the precise role of HSPs in the context of diseases, as they can be present/increased as a cause or a consequence of a disease [15].

HSP27 is a member of the small HSP family and is involved in a multitude of cellular functions such as chaperoning, regulating cell death pathways (e.g. exerting anti-apoptotic effects on cells under stress), cytoskeleton organisation and cell migration [20–22]. HSP27 is expressed in a plethora of tissues e.g. skeletal, cardiac and smooth muscle, brain, spinal cord, kidney, bladder, lung and stomach, however expression levels seem to vary widely [15,23,24]. Altered HSP27 expression has been linked to a variety of conditions including cancer and cardiovascular diseases [25–27]. Recent results on the role of HSP27 in neurodegenerative and neuromuscular diseases suggest a protective role of this chaperone [23]. In contrast, HSP27 expression is increased in several cancer types, e.g. breast, ovarian and prostate cancer, and is associated with worse prognosis and aggressive tumour behaviour [28]. High expression of HSP27 has been observed in metastatic tissue compared to non-metastatic tissue suggesting a role in metastatic spread [29]. The knowledge HSP malfunction in the context of various diseases has led to developing therapeutics targeting HSPs, e.g. HSP27 in treatment of pancreatic, bladder, prostate, ovarian, uterine and colorectal cancer [28]. As HSP27 is ubiquitously expressed it is involved in several physiological and pathological processes in the heart. Increased expression of HSP27 has been observed in exercise induced cardiac hypertrophy, however not pressure overload-induced hypertrophy [30]. Further HSP27 expression doubled in dilated cardiomyopathy patients [31].

The aim of this study was to explore the role of plasma HSP27 in chronic stable HF patients as a predictor of cardiovascular death or unplanned HF associated hospitalisation.

2. Materials and methods

2.1. Patient population

The study was approved by the National Ethics Committee, was conducted according to the declaration of Helsinki and all patients gave written informed consent before enrolment. We prospectively recruited 134 patients from the Heart Failure Outpatient Clinic of the University Clinical Centre Ljubljana as part of the Prognostic Impact of Biomarkers of Inflammation and Coagulation Study [12].

The diagnosis of HF was based on several parameters [12]. Briefly, i) signs/symptoms of HF at inclusion, ii) echocardiographic evidence of left ventricular dysfunction with reduced LVEF (< 40%), mid-range LVEF (40–49%) or preserved LVEF (≥ 50%) with either an E/E′ ratio on tissue Doppler recordings at the mitral ring > 15 or an E/E′ ratio > 8 plus atrial fibrillation, elevated natriuretic peptides or echocardiographic indices of diastolic dysfunction on transmitral and pulmonary veins flow pattern, iii) NYHA functional class II or III, iv) optimal management according to current guidelines and v) stable disease for > 3 months prior to inclusion.

Patients were excluded if they met one of the following criteria: i) < 3 months myocardial infarction, stroke or thromboembolisms with significant liver or renal dysfunction, ii) chronic autoimmune or inflammatory disease or iii) malignancies.

2.2. Study design

At inclusion clinical examination and echocardiographic assessment was performed and venous blood was drawn. Patients were followed for a minimum of 12 months at regular 3 months visits. An event was defined as either cardiovascular death (pump failure, fatal myocardial infarction or sudden cardiac death) or unplanned hospital admission due to worsening of HF (confirmed by two independent cardiologists blinded for baseline measurements).

2.3. Quantification of plasma HSP27 and NT-proBNP

Venous blood samples (EDTA plasma) were taken from all patients. Samples were processed as stated: centrifugation at 3000 rpm for 10 min at 0 °C and plasma was separated immediately and stored at − 80 °C until further use.

We used a commercially available ELISA Kit (DYC1580, R & D Systems, Minneapolis, MN, USA), that was previously compared to other kits and performed best, in this study, to measure total plasma HSP27 concentrations according to the manufacturer's protocol [32]. Briefly, microtitration plates were incubated with a capture antibody. After washing the plates, a blocking step was performed. After another washing step, samples and standards were incubated at defined concentrations. After a further washing step an enzyme linked detection antibody was applied. Wells were washed and incubated with horseradish-peroxidase-conjugate. Again wells were washed and tetramethylbenzidine (TMB; T0440, Sigma-Aldrich Corp., St. Louis, MO, USA) substrate solution was applied to detect enzyme activity. The reaction was stopped with sulphuric acid (1 N). Colour development was measured by Wallac Multilabel Counter 1420 (PerkinElmer, Waltham, MA, USA) at 450 nm. AutoOptical density values obtained at 450 nm (subtracted by plate background measured at 555 nm) were compared to the standard curve calculated from standards with a known concentration of the antigen.

In a previous article we have already validated the R & D HSP27 ELISA Kit we have used for this work. We have evaluated imprecision according to the CLSI guideline EP5-A, within-run and total CV were calculated using the CLSI single-run precision evaluation test. We have analysed three pooled serum samples. The R & D HSP27 ELISA showed a within-run CV of 8.8%, total CV of 12% at a mean serum concentration of 773 pg/ml for pool one; within-in run CV of 6.6%, total CV of 12% at a mean serum concentration of 2699 pg/ml for pool 2; and within-run CV of 5.9%, total CV of 14% at a mean serum concentration of 4509 pg/ml for pool 3. Imprecision of the assay indicated a total CV of < 15% which is appropriate for an ELISA [33].

NT-proBNP was determined with chemiluminescence sandwich