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Plasma levels of alpha-1-antichymotrypsin are elevated in patients with chronic heart failure, but are of limited prognostic value

S. I. Lok · D. J. Lok · P. van der Weide · B. Winkens · P. W. Bruggink-André de la Porte · P. A. Doevendans · R. A. de Weger · P. van der Meer · N. de Jonge

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Abstract

Background There is increasing interest in utilising novel markers of cardiovascular disease risk in patients with chronic heart failure (HF). Recently, it was shown that alpha-1-antichymotrypsin (ACT), an acute-phase protein and major inhibitor of cathepsin G, plays a role in the pathophysiology of HF and may serve as a marker for myocardial distress.

Objective To assess whether ACT is independently associated with long-term mortality in chronic HF patients.

Methods ACT plasma levels were categorised into quartiles. Survival times were analysed using Kaplan-Meier curves and Cox proportional hazards regression, without and with correction for clinically relevant risk factors, including sex, age, duration of HF, kidney function (MDRD), ischaemic HF aetiology and NT-proBNP.

Results Twenty healthy individuals and 224 patients (mean age 71 years, 72 % male, median HF duration 1.6 years) with chronic HF were included. In total, 159 (71 %) patients died. The median survival time was 5.3 (95 % CI 4.5–6.1) years. ACT was significantly elevated in patients (median 433 μg/ml, IQR 279–680) in comparison with controls (median 214 μg/ml, IQR 166–271; p<0.001). Cox regression analysis demonstrated that ACT was not independently related to long-term mortality in chronic HF patients (crude HR=1.03, 95 % CI 0.75–1.41, p=0.871; adjusted HR=1.12, 95 % CI 0.78–1.60, p=0.552), which was confirmed by Kaplan-Meier curves.

Conclusion ACT levels are elevated in chronic HF patients, but no independent association with long-term mortality can be established.

Keywords ACT · Heart failure · Survival

Introduction

Despite recent treatment advances, chronic heart failure (HF) continues to impose a substantial healthcare burden. B-type natriuretic peptide (BNP) and the biologically inactive N-terminal fragment (NT-proBNP) are synthesised by ventricular myocytes in response to haemodynamic stress [1]. Natriuretic peptides (NPs) are useful in determining the diagnosis and prognosis of congestive HF and their use is subsequently advocated by the American College of Cardiology [2] and the European Society of Cardiology guidelines [3]. However, NPs have limitations that affect the interpretation of the results. Elevated NP levels can also be seen in the setting of sepsis [4], acute pulmonary embolism [5] and renal dysfunction [6]. NP levels are higher in women than in men and increase with age [7]. Moreover, NP levels may be reduced in obese patients [8, 9]. Consequently, novel biomarkers are currently under intensive investigation and may be of help to improve the prognostication and clinical outcome of HF patients. Recently, we proposed a role of the acute-phase protein alpha-1-antichymotrypsin (ACT; also
known as SERPINA3) in reverse remodelling [10]. Our previous
data demonstrated that high ACT plasma and myocardial levels
in HF decrease during mechanical support. The goal of this study
was to evaluate the prognostic role of ACT levels with respect to
long-term mortality in chronic HF patients.

Methods and materials

Study population

Patient material consisted of plasma and data obtained from
the Deventer-Alkmaar Heart Failure study (DEAL-HF) [11, 12].
Briefly, 240 patients with typical signs and symptoms of HF
were included, combined with echocardiographic or radionu-
clide ventriculographic findings of a reduced left ventricular
systolic function (LVEF ≤ 45 %) or diastolic dysfunction. The
main exclusion criteria were an expected survival of less than
1 year and planned hospitalisation. In the present study, a com-
plete set of data was available for 224 patients at baseline (due to
missing blood samples). Control plasma was collected from 20
anonymous healthy individuals. The study was approved by the
local Medical Ethics Committees and complied with the Decla-
ration of Helsinki. All patients gave written informed consent.

Laboratory assessment

Routine laboratory measurements and blood samples were
obtained at baseline. EDTA plasma was separated and stored
at minus 70 °C. Circulating levels of ACT were analysed
according to the description of the manufacturer (Genway
Biotech Inc, San Diego, USA). In short, the samples were
diluted 1:5000. Standards and samples were added in duplicate
in a 96-well plate coated with antibody and incubated. After
the first washing step, the conjugate was added, followed by
incubation. The next washing step was followed by the addi-
tion of the substrate solution and incubation. The stop solution
was added and wells were read out on a microplate reader.

Clinical follow-up

ACT values were assessed for all-cause mortality. Patients
were followed up to 10.5 years after randomisation at the
outpatient clinic. In case of no show, information regarding
survival was obtained from the hospital system, relatives or
general practitioner.

Statistics

Categorical data are presented by number (%) and numerical
data by mean±standard deviation or by median (interquartile
range, IQR, i.e. 25th–75th percentile), where appropriate. Com-
parisons between patients and healthy controls were performed
using independent-samples t-test or Mann-Whitey U-test for
numerical variables and Chi-square or Fisher’s exact test for
categorical variables. Linear regression analysis was performed
to assess clinically relevant factors independently related to ACT
plasma levels, such as sex, age, duration of HF, kidney function
(MDRD), ischaemic HF aetiology and NT-proBNP. Survival
times were analysed using Kaplan-Meier curves and Cox pro-
portional hazards (PH) regression, without and with correction
for the above-mentioned clinically relevant risk factors. Time to
event was defined as time between inclusion and death or to end
of study/loss to follow-up (censored). PH assumption was
checked using Schoenfeld residuals and linearity assumption
by adding and testing mean-centred quadratic terms. A p-value
≤ 0.05 was considered to be statistically significant. All analyses
were done with SPSS 20.0 software (SPSS Inc, Chicago, IL).

Results

Baseline characteristics

Characteristics of the study population are described in Table 1.
The study cohort consisted of patients with severe chronic HF
with a mean age of 71 years, 72 % were male with a median HF
duration of 1.6 years. Almost all patients (97 %) had left ventric-
ular systolic dysfunction with a reduced ejection fraction, the
mean ejection fraction being 31 %. At the time of inclusion,
ischaemic aetiology of HF was present in 146 patients (65 %).
Non-survivors were older, male subjects with a longer duration of
HF, higher C-reactive protein and NT-proBNP levels, more often
had kidney dysfunction, were more often diagnosed with diabetes
mellitus, anaemia and ischaemic heart disease and were less often
treated with beta-blocking agents in comparison with survivors.

Plasma levels of ACT in patients and healthy controls

Figure 1 shows the plasma levels of ACT in patients and
healthy controls. A large individual variation of ACT was
found. ACT was significantly elevated in patients (median
433 μg/ml, IQR 279–680) in comparison with controls
(median 214 μg/ml, IQR 166–271; p < 0.001). A linear
regression analysis showed that the duration of HF was inde-
dependently related to ACT plasma levels (patients with a shorter
duration of HF had a higher ACT plasma level; p = 0.031).
Only 5.4 % of the variation in ACT plasma levels is explained
by the model (R-square = 0.054), which means that the large
differences in ACT plasma levels between patients cannot be
explained by the variables included in the model.

ACT levels and mortality

The mean and median survival time was 5.5 and 5.3 years,
respectively. In total, 159 (71 %) patients died. Cox-proportional
hazard regression models showed that ACT plasma levels (mg/ml) were not significantly related to long-term mortality (crude HR=1.03, 95 % CI 0.75–1.41, \( p =0.871 \); adjusted HR=1.12, 95 % CI 0.78–1.60, \( p =0.552 \)). HR present the effect of ACT per 1000 μg/ml=1 mg/ml. This non-significant effect of ACT was also confirmed by the Kaplan-Meier curves presented in Fig. 2, where circulating ACT was divided into quartiles and presented as Kaplan-Meier curves.

### Discussion

In the present study, ACT levels were significantly elevated in chronic HF patients in comparison with healthy controls and demonstrated that a single measurement of plasma ACT is not an independent risk factor for long-term mortality in these patients.

Multiple microarray analyses have been conducted to screen the gene expression profile of the failing myocardium from patients with dilated cardiomyopathy and suggested elevated ACT expression in the failing heart [13–16]. These findings were corroborated by our previous work in a small panel of severe end-stage HF patients who demonstrated profound elevated ACT levels in heart tissue as well as plasma at the time of left ventricular assist device implantation. The present study aimed to verify ACT up-regulation in a larger cohort of chronic HF patients.

ACT seems to be involved in the pathophysiology of HF. The exact role of ACT in HF is unknown, but several effects...
on the cardiovascular system have been postulated. ACT is a serine protease inhibitor, mainly of cathepsin G [17]. By eliminating cathepsin G, ACT might prevent the degradation of connective tissue proteins [18] and the activation of the transforming growth factor pathway [19], with subsequently less cardiomyocyte necrosis, hypertrophy and fibrosis. Also, ACT is an acute-phase protein and induces tumour necrosis factor (TNF)-α and NF-κB [20]. Additionally, ACT is thought to be protective during ischaemia reperfusion by inhibiting neutrophil accumulation into the ischaemic-reperfused myocardium and by inactivating cytotoxic metabolites released from neutrophils [21].

The present study demonstrated that ACT plasma levels were elevated in chronic HF patients, suggesting that ACT might be useful as a diagnostic marker in HF. Nevertheless, the present Cox PH regression analyses demonstrated that ACT is not an independent risk factor for long-term mortality in these patients with severe chronic HF. Future studies with plasma samples taken at different time points and taken from patients with less severe HF are necessary to analyse the potential role of ACT as a prognostic marker in HF.

**Limitations**

The number of patients after 10 years of follow-up was small. As a sensitivity analysis, data up to 5 years of follow-up were also analysed with Cox regression, and showed similar results. Only one random plasma sample per patient was available for the present study. As a result, the possible effect of a change in levels of ACT on mortality is unknown. In this study, mainly chronic HF patients with an older age and reduced ejection fraction (HFrEF) were included. Our results cannot be extrapolated to young patients and/or less severe forms of HFrEF, acute HF and HF with a preserved left ventricular systolic function (HFpEF). The main exclusion criteria, expected survival of less than 1 year and planned hospitalisation, may have caused a selection bias, since these patients are expected to have high ACT levels. The endpoint of the present study was all-cause mortality. Therefore, we are not informed about the relation between the novel marker and hospitalisations for HF or other serious events.

**Conflicts of interest** None declared.

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