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Triple head-to-head comparison of fibrotic biomarkers galectin-3, osteopontin and gremlin-1 for long-term prognosis in suspected and proven acute heart failure patients

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ABSTRACT

Background: To comparatively evaluate long-term prognostic values of fibrotic biomarkers galectin-3, gremlin-1 and osteopontin in patients presenting to the emergency department (ED) suspected of acute heart failure (AHF).
Methods: Patients with acute dyspnea or peripheral edema were enrolled in the ED. Biomarkers were measured and added to prognostic models including 11 conventional risk factors plus NT-proBNP assessing state-of-the-art statistics of discrimination, calibration, reclassification and Cox regression analyses. Prognostic outcomes were long-term all-cause mortality (ACM) and AHF-related rehospitalization (AHF-RH) at 1 and 5 years.
Results: 401 patients including 122 AHF patients were enrolled (mean age 67 years, males 51%). During 5 years follow-up 129 patients (30%) died and 73 (18%) were re-hospitalized because of AHF. In multivariate analysis, galectin-3 (hazard ratios (HR) range 1.4–1.9; $p = 0.03$) and osteopontin (HR range 1.2–1.4; $p = 0.001$) remained associated with ACM overall and in the AHF population at 5 years, whereas gremlin-1 remained associated with AHF-RH at 1 year in AHF patients (HR 1.3; $p = 0.002$). ACM in whole cohort was best discriminated (AUC = 0.85, $p = 0.0001$), calibrated and re-classified (NRI +0.50 to +0.56, $p = 0.0001$) by galectin-3, whereas in AHF patients ACM was best discriminated by osteopontin (AUC range: 0.82–0.84, $p = 0.0001$; NRI +0.34 to +0.38, $p < 0.1$) and AHF-RH at 1 year by gremlin-1 (AUC range: 0.82–0.92, $p = 0.0001$; NRI +0.59 to +0.60, $p = 0.006$).
Conclusions: A panel of fibrotic biomarkers, including osteopontin, galectin-3 and gremlin-1, might be useful for long term risk-stratification of symptomatic ED patients being suspected of AHF.

1. Introduction

In Western population the increasing and age-dependent overall prevalence of heart failure is estimated to be 1–2% [1]. A decrease of heart failure related mortality was achieved by improving effective diagnostics and therapies over the last decades [1]. In contrast, patients with an unstable course of disease require frequent, unplanned presentation at the emergency department (ED) and consecutive rehospitalization [2]. Despite symptoms´ relief frequent rehospitalizations are proven to be associated with a severe relapse, loss of quality of life as well as with extensive costs for health care systems [3].

Circulating biomarkers have entered the field of heart failure research aiming to improve diagnostics, to guide medical therapies and to optimize risk stratification [4]. Natriuretic peptides are considered as standard biomarkers for diagnostic and prognostic risk assessment in patients suffering from acute heart failure (AHF) [1]. However, natriuretic peptides represent compensatory cardiac hormones being synthesized as a consequence of the failing heart [5]. Increasing scientific interest has emerged for biomarkers reflecting the causative pathomechanisms regarding heart failure development, such as myocardial ischemia, cardiac inflammation [6] or cardiac fibrosis being associated with an adverse structural remodeling [7].

Commonly novel cardiac biomarkers for AHF were assessed within different study sites and heterogeneous populations [4]. On the

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contrary, biomarkers reflecting only one heart failure etiology are rarely investigated in one single representative cohort [4]. The latter comparative methodological approach might however reveal both strengths and weaknesses of biomarkers reflecting a single pathophysiological etiology in advance [8]. Accordingly, the long-term prognostic values of biomarkers reflecting the potential development of causative cardiac fibrosis, such as galectin-1, ostopeontin and gremlin-1, have never been comparatively evaluated within one study cohort of patients suspected to suffer from AHF [9–11].

Therefore, it was hypothesized that additional measurements of these fibrotic biomarkers may correlate with long-term prognosis regarding specifically all-cause mortality (ACM) and AHF-related rehospitalization (AHF-RH) in these patients. In order to prove this hypothesis the present study aims to evaluate a triple head-to-head comparison of these fibrotic biomarkers for long-term acute heart failure prognosis in patients presenting to the ED with symptoms of acute dyspnea or peripheral edema being suspected to suffer from AHF.

2. Materials and methods

2.1. Study patients, design and data collection

The present study represents a post hoc analysis of a specimen repository from patients enrolled in the Mannheim NT-proBNP Study (MANPRO, clinicaltrials.gov identifier: NCT00143793), which was conducted as a mono-centric prospective controlled study at the University Medical Centre Mannheim (UMM), Germany. Within our University Clinic comprehensive heart failure treatment is well established and universally applied to all patients, continuously including and adapting current guidelines and newest innovations of heart failure treatment with regard to medication, interventional and device therapies (i.e. 24 h catheterization laboratory, invasive electrophysiologic studies, implantation of ICDs, cardiac resynchronization therapy, cardiac contractility modulation and further modern devices). The original objective of the MANPRO study was to determine the diagnostic power of a formerly newly-developed NT-proBNP assay to identify patients with AHF and to evaluate the economic consequences of including this assay in symptomatic ED patients with acute dyspnea and/or peripheral edema being suspected of AHF. It was shown that including initial measurements of NT-proBNP in the ED setting improved diagnostic pathways and resource allocation, as previously being published [12]. The study was carried out according to the principles of the declaration of Helsinki and was approved by the medical ethics commission II of the Faculty of Medicine Mannheim, University of Heidelberg, Germany. Informed consent was obtained from all participating patients or their legal representatives.

Inclusion criteria of the MANPRO study were patients with symptoms of acute dyspnea and/or peripheral edema presenting in the ED, which were consecutively included from August 2005 until March 2006. Patients suffering from severe renal disease (defined as serum creatinine level greater than 2.8 mg/dl), anemia (hemoglobin concentrations below 8.0 g/dl), obvious traumatic causes of dyspnea, pregnancy, with a status after immediate cardiopulmonary resuscitation, participation in another clinical trial and patients with age under 18 years were excluded [12].

2.2. Diagnosis of acute heart failure

The investigators of the study were neither involved in therapeutic decisions nor in decisions regarding clinical examinations. An independent study physician being blinded to the results of biomarker measurements but having unrestricted access to all clinical patient data classified all patients into two categories: 1) Symptomatic patients because of AHF, 2) symptomatic patients due to any cause except for AHF. Diagnosis of AHF was based on European Guidelines for the diagnosis of AHF [1,13]. Detailed diagnostic criteria being applied on our study population have previously been published [9,12]. Patients were classified according to the functional NYHA classification and structural ABCD classification of the American College of Cardiology/American Heart Association [1]. Standard two-dimensional and color Doppler imaging was performed by independent cardiologists during routine clinical care to assess standard echocardiographic indices, such as left ventricular (LV) systolic function comprising Simpson's biplane left ventricular ejection fraction (LVEF) [35].

2.3. Measurements of gremlin-1, osteopontin, galectin-3 and NT-proBNP

All samples were obtained by venipuncture into serum and ammonium heparin tubes for biomarker measurements, immediately at presentation to the ED. Within 30 min all blood samples were centrifuged at 2000 *g* for 10 min. Plasma and serum were separated, aliquoted, frozen and stored at −80 °C.

Blood samples for biomarker measurements were available in all patients. Biomarker measurement was performed by commercially available enzyme-linked immunosorbent assays: osteopontin (Quantikine Human Osteopontin (OPN) Immunoassay (R&D Systems Inc., Minneapolis, USA) [9], gremlin-1 (Human Grem1 ELISA®, USCN Life Science Inc., Wuhan, China) [14], galectin-3 (Galectin 3, Architect System, Abbott, Wiesbaden, Germany) [15] and NT-proBNP (Flex reagent cartridge PBNP, Dimension System, Dade Behring Ltd., Atterbury, Milton Keynes, UK) as previously described [12]. The measuring range (linear) for galectin-3 was 4.0 -114 μg/l with a detection limit of 1 μg/l. The measuring range for gremlin-1 was 0.47–30 μg/l with a detection limit of 0.15 μg/l. The measuring range for osteopontin in healthy individuals was 53.4–195 ng/ml with a test specific detection limit of 0.011 ng/mL.

Osteopontin and NT-proBNP were measured from aliquots of ammonium heparin monovettes®, whereas galectin-3 and gremlin-1 were measured from aliquots of serum monovettes® in all 401 patients.

2.4. Prognostic endpoints

Two prognostic outcomes were considered: ACM and AHF-RH at 1 and 5 years. Follow-ups were performed in three successive steps using our in-hospital electronic records, contacting family physicians and performing individual telephone visits. The rate of complete lost to follow-up with regard to survival status was 2.2% after 1 year and 11.2% after 5 years, as previously reported [9]. Follow-up with regard to AHF-RH at index-hospital was completed in all study patients, while AHF-RH in other hospitals was not documented [9].

2.5. Statistical methods

The Student *t*-test was applied for normally distributed data. Otherwise, the Mann–Whitney *U* test was used as nonparametric test. Deviations from a Gaussian distribution were tested by the Kolmogorov–Smirnov test. NT-proBNP data were $\log_{10}$ transformed, thereby promoting normality, and the unpaired *t*-test was applied. Spearman's rank correlation for nonparametric data was used to test univariate correlations. Qualitative parameters were analyzed using a 2×2 contingency table and Chi^2 test or Fisher's exact test as appropriate. Quantitative data are presented as mean ± standard error of mean (SEM) or as median and interquartile range (IQR), depending on the distribution of the data. For qualitative parameters absolute and relative frequencies are presented. All analyses were exploratory and utilized a p value of <0.05 (2 tailed) for significance, and a p value of <0.1 (2 tailed) for statistical trend.

2.6. Prognostic value of gremlin-1, osteopontin, and galectin-3

Kaplan–Meier curves were created according to gremlin-1 and galectin-3 quartiles and the corresponding hazard ratios (HRs) were