CHAPTER 2

The Fibrosis Marker Syndecan-1 and Outcome in Heart Failure Patients with Reduced and Preserved Ejection Fraction

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Circulation: Heart Failure. 2014 May;7(3):457-62
ABSTRACT

Background: Syndecan-1 is a member of the proteoglycan family involved in cell-matrix interactions. Experimental studies showed that syndecan-1 is associated with inflammation in acute myocardial infarction and remodeling. The goal of this study was to explore the role of syndecan-1 in human heart failure (HF).

Methods: We analyzed plasma syndecan-1 levels in 567 patients with chronic HF. Primary endpoint was a composite of all-cause mortality and re-hospitalization for HF at 18 months.

Results: Mean age was 71.0±11.0 years, 38% was women, and mean LVEF was 32.5±14.0%. Median syndecan-1 levels were 20.1 ng/mL (IQR 13.9-27.7 ng/mL). Patients with higher syndecan-1 levels were more often men, had higher N-terminal probrain-type natriuretic peptide levels and worse renal function. Multivariable regression analyses showed a positive correlation between syndecan-1 levels and fibrosis and remodeling but no correlation with inflammation markers. Interaction analysis revealed an interaction between LVEF and syndecan-1 (p=0.047). A doubling of syndecan-1 was associated with an increased risk of the primary outcome in patients with HF with preserved ejection fraction (hazard ratio: 2.10, 95%confidence interval [1.14-3.86]; p=0.017) but not in patients with HF with reduced ejection fraction (hazard ratio: 0.95, 95%confidence interval [0.71-1.27]; p=0.729). Finally, syndecan-1 enhanced risk classification in patients with HF with preserved ejection fraction when added to a prediction model with established risk factors.

Conclusion: In patients with HF, syndecan-1 levels correlate with fibrosis biomarkers pointing towards a role in cardiac remodeling. Syndecan-1 was associated with clinical outcome in patients with HF with preserved ejection fraction but not in patients with HF with reduced ejection fraction.

ABBREVIATIONS

COACH: Coordinating study evaluating Outcomes of Advising and Counseling in Heart Failure
CRP: C-reactive protein
eGFR: estimated glomerular filtration rate
ELISA: enzyme-linked immunosorbent assay
HF: heart failure
HFpEF: heart failure with a preserved ejection fraction
HFrEF: heart failure with a reduced ejection fraction
IL-6: interleukin-6
LVEF: left ventricular ejection fraction
MDRD: modification of diet in renal disease
MMP: matrix metalloproteinase
TGF-β: transforming growth factor beta
TIMP: tissue inhibitors of metalloproteinase
INTRODUCTION

Extracellular matrix components, particularly proteoglycans, are associated with inflammation, fibrosis and cardiac remodeling (1). Members of the syndecan family have been found to be associated with the onset of cardiac fibrosis by functioning as an important target for transforming growth factor-β (2, 3). Experimental studies in mice have shown that syndecan-1 was involved in both inflammation and fibrosis after myocardial injury (2–4). Syndecan-1 had a protective effect in short-term inflammation post-myocardial infarction resulting in less remodeling through direct ECM involvement in wound healing (3, 4). However, in the long term it might lead to increased fibrosis and remodeling through the involvement of activated RAAS stimulation (2). The ecto-domain of the transmembrane receptor syndecan-1 protein has been known to shed into the extracellular matrix; consequentially the ecto-domain of syndecan-1 is measurable in plasma (5). We recently reported sex-specific differences in biomarker levels in heart failure (HF) patients related to inflammation and fibrosis (6). We therefore hypothesized that syndecan-1 might be associated with fibrosis and adverse outcome in patients with HF. In the present study we aimed to further establish the association between syndecan-1 and markers of inflammation and fibrosis, and assess the prognostic value of syndecan-1 in patients with HF with preserved and reduced left ventricular ejection fractions.

METHODS

Patient population and study design

The current study was performed as a sub-study of the Coordinating study evaluating Outcomes of Advising and Counseling in HF (COACH). In brief, 1023 patients were included to participate in a prospective randomized disease management study. The rationale and outcomes of this trial have been reported elsewhere (7–9). Both patients with HF with preserved ejection fraction (HFpEF) and reduced ejection fraction (HFrEF) were included in the study. The cut-off point of left ventricular ejection fraction (LVEF) to identify HFpEF was predefined at >40% in the study protocol, and similar to a previously published study from this cohort (9). Samples for biomarker analysis were obtained from a subset of 567 patients, who were representative for the entire study population regarding baseline characteristics. Prior to discharge, when patients were stabilized after an acute HF admission, samples were collected. In 460 patients, measurement of LV function was performed at discharge. This study complies with the Declaration of Helsinki, local medical ethics committees approved the study, and all patients provided written informed consent.

Endpoints

The primary endpoint in this study was defined as the combined end-point of all-cause mortality or re-hospitalization at 18 months, where re-hospitalization was defined as an unplanned overnight
hospital stay connected to worsening HF. The secondary endpoint was defined as all-cause mortality at 3 years. All events were evaluated and adjudicated by an independent end-point committee.

**Biochemical Analysis**

Prior to discharge fibrosis markers including syndecan-1, galectin-3, periostin and ST-2 were measured, using a commercially available competitive enzyme-linked immunosorbent assay (ELISA) (Alere San Diego Inc. (San Diego, CA, USA)). Measurements were made with the usage of the luminex platform. Lower limits for the detection of syndecan-1 with this specific ELISA were 2.4 ng/ml; intra- and interassay coefficients of variation are 25% and 25% respectively. Interleukin-6 (IL-6), C-reactive protein (CRP) and Transforming Growth Factor beta one (TGF-β1) were measured in a 96-well polystyrene microtiter plate using searchlight proteome arrays, as previously described (10, 11). Measurement of N-terminal pro-brain-type natriuretic peptide (NT-proBNP) was done using the Elecsys proBNP ELISA, (Roche diagnostics, Mannheim, Germany). Estimate glomular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula (12).

**Statistical analysis**

Data are expressed as means ± SD when normally distributed, as medians with lower and upper quartiles when non-normally distributed or as numbers and percentages when categorical. Baseline characteristics were divided into quartiles of syndecan-1. Intergroup differences were tested using the 1-way analysis of variance test, Kruskal-Wallis test, or Pearson χ² test when appropriate. For further analyses, skewed variables were transformed to a 2-log scale to achieve a normal distribution. Risk estimates for the transformed variables should be interpreted as the relative risk if values were doubled (e.g. 2 to 4 mmol/L).

To establish clinical determinants of syndecan-1 levels and its relation to other markers of inflammation and fibrosis, multiple linear regression models were constructed. Variables with a significant univariate association with syndecan-1 (< 0.10) were entered in a stepwise backward multivariate model based on the strength of their univariate association.

Univariate and multivariate Cox proportional hazard regression models were used to calculate the predictive value of syndecan-1 on both the primary and secondary endpoint. In 2 consecutive multivariable models, syndecan-1 was adjusted for age, sex, the presence of diabetes, previous HF hospitalizations, LVEF, renal function, levels of NT-proBNP and finally for galectin-3, periostin, ST-2 levels and a history of MI.

Finally, risk stratification of syndecan-1 levels on top of the COACH risk engine model, as described elsewhere, was tested for both endpoints using the continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) (13). As suggested, the continuous NRI is a more objective and versatile measure of improvement in risk prediction compared to the categorical NRI (14). Variables in the COACH risk model include: age, sex, BMI, blood pressure, pulse pressure, a prior stroke and/or MI, previous HF hospitalizations, the presence of peripheral artery
disease, atrial fibrillation and/or diabetes, renal function, and levels of NT-proBNP and sodium. All tests were 2 sided, and a P value < 0.05 was considered statistically significant. All statistical analyses were performed using STATA version 11.0 (StataCorp LP, College station, Texas, USA).

RESULTS

Patient characteristics

Baseline characteristics are described in Table 1. Of the 567 patients, 38% was female, 47 percent was in NYHA class II and 49 percent in NYHA class III. Mean left ventricular ejection fraction (LVEF) was measured in 460 patients prior to discharge and was 32.5 ± 14.0%. Patients with higher syndecan-1 levels were more often male, had lower blood pressures, a lower LVEF and more previous HF related hospitalizations. Additionally, higher levels of NT-proBNP, fibrosis markers and a worse renal function were observed in patients with higher syndecan-1 levels. Interestingly, no elevated levels of inflammatory markers were observed in patients with higher syndecan-1 levels.

Table 1. Baseline characteristics of all 567 patients at discharge, divided into quartiles of syndecan-1 (ng/mL)

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n = 567)</th>
<th>Q1 (n = 141)</th>
<th>Q2 (n = 143)</th>
<th>Q3 (n = 142)</th>
<th>Q4 (n = 141)</th>
<th>p-value (trend)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syndecan-1, min-max (ng/mL)</td>
<td>2.4 - 393.0</td>
<td>2.4 - 13.9</td>
<td>14.0 - 20.1</td>
<td>20.2 - 27.6</td>
<td>27.7 - 393.0</td>
<td>NA</td>
</tr>
<tr>
<td>Demographics and clinical signs</td>
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</tr>
<tr>
<td>Age (years)</td>
<td>71.0 ± 11.0</td>
<td>70.3 ± 11.5</td>
<td>70.6 ± 11.5</td>
<td>72.3 ± 9.7</td>
<td>71.0 ± 11.0</td>
<td>0.544</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>38.1</td>
<td>48.2</td>
<td>39.2</td>
<td>31</td>
<td>34</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 (23.5 - 29.5)</td>
<td>26.7 (24.0 - 29.9)</td>
<td>26.8 (23.9 - 30.1)</td>
<td>25.9 (23.9 - 29.0)</td>
<td>25.8 (23.1 - 29.4)</td>
<td>0.079</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>118.2 ± 21.2</td>
<td>122.2 ± 23.2</td>
<td>120.4 ± 20.4</td>
<td>116.7 ± 20.4</td>
<td>113.3 ± 19.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>74.3 ± 13.1</td>
<td>74.6 ± 12.2</td>
<td>73.8 ± 12.3</td>
<td>75.0 ± 15.6</td>
<td>73.8 ± 11.7</td>
<td>0.355</td>
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<tr>
<td>LVEF (%)</td>
<td>32.5 ± 14.0</td>
<td>33.0 ± 13.8</td>
<td>34.2 ± 13.8</td>
<td>32.6 ± 14.7</td>
<td>30.0 ± 13.4</td>
<td>0.036</td>
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<td>Previous HF hospitalization</td>
<td>34.4</td>
<td>28.4</td>
<td>32.2</td>
<td>35.9</td>
<td>41.1</td>
<td>0.026</td>
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<tr>
<td>NYHA class, II/III/IV (%)</td>
<td>46.6/49.8/3.6</td>
<td>56.8/39.6/3.6</td>
<td>41.3/57.3/1.4</td>
<td>43.9/51.8/4.3</td>
<td>44.7/50.3/5.0</td>
<td>0.087</td>
</tr>
<tr>
<td>Medical history (%)</td>
<td></td>
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<tr>
<td>Myocardial infarction</td>
<td>40.9</td>
<td>35.5</td>
<td>39.8</td>
<td>43.7</td>
<td>44.7</td>
<td>0.11</td>
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<tr>
<td>Stroke</td>
<td>15.3</td>
<td>12.1</td>
<td>21.7</td>
<td>16.9</td>
<td>10.6</td>
<td>0.524</td>
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<td>Hypertension</td>
<td>42.3</td>
<td>37.6</td>
<td>51.1</td>
<td>38</td>
<td>42.6</td>
<td>0.876</td>
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<tr>
<td>Atrial fibrillation of flutter</td>
<td>46</td>
<td>38.3</td>
<td>47.6</td>
<td>48.6</td>
<td>49.7</td>
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<td>Diabetes</td>
<td>30.5</td>
<td>32.2</td>
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<td>27.5</td>
<td>31.9</td>
<td>0.762</td>
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<td>COPD</td>
<td>28</td>
<td>29.1</td>
<td>21</td>
<td>33.8</td>
<td>28.4</td>
<td>0.606</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.1 ± 2.0</td>
<td>13.3 ± 2.2</td>
<td>13.2 ± 2.1</td>
<td>13.0 ± 1.9</td>
<td>13.0 ± 1.8</td>
<td>0.533</td>
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<tr>
<td>Sodium (mmol/L)</td>
<td>139 ± 4</td>
<td>139 ± 4</td>
<td>139 ± 4</td>
<td>139 ± 5</td>
<td>138 ± 4</td>
<td>0.142</td>
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Predictors of syndecan-1 levels in heart failure

To assess whether syndecan-1 was associated with fibrosis or inflammation, a multivariable regression analysis was performed as shown in Table 2. A clear positive association was found relating to fibrotic and remodeling markers, including peristin, galectin-3 and ST-2 (all \( p < 0.001 \)). No correlation could be observed between syndecan-1 and the inflammatory markers hs-CRP (\( p = 0.635 \)) and IL-6 (\( p = 0.838 \)). A negative correlation was observed between syndecan-1 and renal function (\( p = 0.009 \)). Furthermore, sex was found to be a predictor of syndecan-1 levels (\( p = 0.029 \)).
Table 2. Clinical variables associated with syndecan-1 (per doubling) in chronic heart failure

<table>
<thead>
<tr>
<th>Variables</th>
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<th></th>
<th>Multivariate</th>
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<td>p-value</td>
<td>Beta</td>
<td>p-value</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>-0.008</td>
<td>0.854</td>
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<td>Female sex (%)</td>
<td>-0.092</td>
<td>0.027</td>
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<td>-0.125</td>
<td>0.029</td>
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<td>BMI (kg/m²)</td>
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<td>0.02</td>
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<td>Systolic BP (per 5 mmHg)</td>
<td>-0.141</td>
<td>0.001</td>
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<td>Heart rate (bpm)</td>
<td>-0.058</td>
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<td>LVEF (%)</td>
<td>-0.054</td>
<td>0.249</td>
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<td>Previous HF hospitalization</td>
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<td>0.031</td>
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<td>NYHA class, II/III/IV (%)</td>
<td>0.085</td>
<td>0.043</td>
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<td>Medical history (%)</td>
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<tr>
<td>Myocardial infarction</td>
<td>0.039</td>
<td>0.358</td>
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<tr>
<td>Stroke</td>
<td>-0.03</td>
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<td>Hypertension</td>
<td>-0.002</td>
<td>0.963</td>
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<td>Atrial fibrillation</td>
<td>0.087</td>
<td>0.037</td>
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<tr>
<td>Diabetes</td>
<td>-0.021</td>
<td>0.619</td>
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<tr>
<td>COPD</td>
<td>0.013</td>
<td>0.765</td>
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<tr>
<td>Laboratory</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>0.024</td>
<td>0.67</td>
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<tr>
<td>Sodium (mmol/L)</td>
<td>-0.048</td>
<td>0.259</td>
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<tr>
<td>NT-proBNP (per doubling)</td>
<td>0.23</td>
<td>&lt; 0.001</td>
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<tr>
<td>high-sensitive CRP (per doubling)</td>
<td>0.021</td>
<td>0.635</td>
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<td>IL-6 (per doubling)</td>
<td>0.009</td>
<td>0.838</td>
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<td>ST-2 (per doubling )</td>
<td>0.622</td>
<td>&lt; 0.001</td>
<td></td>
<td>0.23</td>
<td>&lt; 0.001</td>
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<tr>
<td>Galectin-3 (per doubling)</td>
<td>0.499</td>
<td>&lt; 0.001</td>
<td></td>
<td>0.357</td>
<td>&lt; 0.001</td>
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<tr>
<td>Periostin (per doubling)</td>
<td>0.634</td>
<td>&lt; 0.001</td>
<td></td>
<td>0.516</td>
<td>&lt; 0.001</td>
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<tr>
<td>TGF-beta (per doubling)</td>
<td>-0.024</td>
<td>0.573</td>
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<tr>
<td>eGFR (per 5 mL/min/1.73m²)</td>
<td>-0.111</td>
<td>0.009</td>
<td>-0.027</td>
<td>&lt; 0.001</td>
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<tr>
<td>BUN (per doubling)</td>
<td>0.112</td>
<td>0.011</td>
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<tr>
<td>Treatment at discharge (%)</td>
<td></td>
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<td>ACE inhibitor or ARB</td>
<td>-0.042</td>
<td>0.312</td>
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<td>Beta blocker</td>
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<td>0.018</td>
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<td>Diuretic</td>
<td>0.073</td>
<td>0.081</td>
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<td>MRA</td>
<td>-0.009</td>
<td>0.833</td>
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<td>Statin</td>
<td>-0.015</td>
<td>0.718</td>
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<td>Digoxin</td>
<td>0.008</td>
<td>0.845</td>
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</tbody>
</table>

Values are standardized beta coefficients. Abbreviations: ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HF, heart failure; IL-6, Interleukin 6; LVEF, left ventricular ejection fraction; MRA, Mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; NYHA, New York Heart Association; TGF-beta, transforming growth factor beta; sTNFR-1, soluble tumor necrosis factor receptor 1.
Syndecan-1 & clinical outcome in heart failure

After 18 months, 240 patients reached the combined endpoint and 234 patients died after 3 years. In univariable analysis, a doubling of syndecan-1 levels showed a significant increase risk for both the combined endpoint (hazard ratio (HR): 1.20, 95% confidence interval (CI) [1.05 - 1.37]; p = 0.005) and for all-cause mortality after 3 years (HR: 1.27, 95% CI [1.12-1.44]; p<0.001) (Table 3). However, when adjusting for age, sex, presence of diabetes, previous HF hospitalizations, LVEF, renal function and NT-proBNP, syndecan-1 was no longer significantly associated with both endpoints.

Table 3. Hazard ratios in predicting the combined endpoint (HF hospitalizations or all-cause mortality at 18 months) or all-cause mortality at 3 years in overall HF and divided into HFrEF and HFpEF

<table>
<thead>
<tr>
<th>Syndecan-1 (per doubling)</th>
<th>Combined endpoint</th>
<th>All cause mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Overall HF (n = 567)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Univariate</td>
<td>1.20 (1.05 - 1.37)</td>
<td>0.005</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.21 (1.06 - 1.40)</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.08 (0.91 - 1.28)</td>
<td>0.385</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.08 (0.84 - 1.39)</td>
<td>0.563</td>
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<tr>
<td>HFrEF (n = 353)</td>
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<tr>
<td>Univariate</td>
<td>1.12 (0.95 - 1.33)</td>
<td>0.18</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.12 (0.94 - 1.33)</td>
<td>0.223</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.98 (0.80 - 1.21)</td>
<td>0.901</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.95 (0.71 - 1.27)</td>
<td>0.729</td>
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<tr>
<td>HFpEF (n = 107)</td>
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<tr>
<td>Univariate</td>
<td>1.30 (1.05 - 1.61)</td>
<td>0.016</td>
</tr>
<tr>
<td>Model 1</td>
<td>1.33 (1.07 - 1.66)</td>
<td>0.009</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.37 (1.01 - 1.86)</td>
<td>0.046</td>
</tr>
<tr>
<td>Model 3</td>
<td>2.10 (1.14 - 3.86)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Model 1 is adjusted for age en sex. Model 2 is adjusted for model 1 + presence of diabetes, previous HF hospitalizations, LVEF (only in overall HF), renal function and NT-proBNP levels. Model 3 is adjusted for model 2 + levels of Galectin-3, ST2 and periostin and prior MI. Abbreviations: CI, confidence interval; HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HR, hazard ratio.

Interaction analysis showed an interaction between syndecan-1 and LVEF for both the combined endpoint (p = 0.047) and 3-years mortality (p = 0.003). Consequently, patients were sub-divided into those with preserved LVEF (n = 107) and reduced LVEF (n = 353). Within these subgroups, 143 patients with HFrEF and 50 patients with HFpEF reached the primary combined end point at 18 months. Furthermore, 142 patients with HFrEF and 44 patients with HFpEF reached the secondary end point at 3 years. The interaction between HFrEF, HFpEF and syndecan-1 levels is shown in Figure 1. This figure depicts how an increase in syndecan-1 levels pose a much stronger increase in risk for patients with HFpEF than in patients with HFrEF (Figure 1). There was no
interaction between syndecan-1, sex and the primary endpoint (p = 0.232). A significant interaction was found for syndecan-1 and gender for the secondary endpoint (p=0.017). When subdividing patients with HFrEF by sex, a significant predictive value was found for syndecan-1 levels in female patients with HFrEF (HR, 1.08; 95% CI, 0.52–2.25; P=0.843). Syndecan-1 was not associated with an increased risk for either the primary end point (HR, 0.95; 95% CI, 0.71–1.27; P=0.729) or the secondary end point (HR, 1.11; 95% CI, 0.83–1.78; P=0.477) in patients with HFrEF (Table 3). A strong predictive value was found for doubling of syndecan-1 in patients with HFrEF for the combined end point (HR, 1.30; 95% CI, 1.05–1.61; P=0.016) and for 3-year mortality (HR, 1.52; 95% CI, 1.22–1.90; P<0.001; Table 3). This association remained statistically significant in the multivariable corrected model for both the combined endpoint (HR: 2.10, 95%CI [1.14-3.86]; p = 0.017) and 3-year mortality (HR: 2.00, 95%CI [1.01-3.98]; p = 0.044).

Finally, NRI and integrated discrimination improvement showed a significant additive value for

**Figure 1.** Graphical depiction of the risk estimates for the primary endpoint in patients with HFrEF versus HFrEF. The distribution of (log2-transformed) syndecan-1 is depicted in grey bars in the background.

the combined primary end point in patients with HFrEF, when syndecan-1 was added on top of variables of the COACH risk engine model. This additive value was not observed in patients with HFrEF (Table 4).
**DISCUSSION**

This study aimed to extend the knowledge of syndecan-1 plasma levels by assessing the role of syndecan-1 in HF patients. The findings of this study have demonstrated that syndecan-1 is associated with fibrotic and remodeling markers galectin-3, periostin and ST-2, whereas no correlation with inflammation markers was observed, confirming earlier published experimental *in vitro* results in a human clinical setting (2). In addition, this study identified syndecan-1 as a specific predictor for clinical outcome in HFpEF, but not in HFrEF patients.

Syndecan-1 is a heparan-sulfate proteoglycan that functions as an important cell receptor in the extracellular matrix and is found on the cell surfaces of almost all cell types. As such, it is involved in a wide array of processes in human (patho)physiology (15). Animal models showed that syndecan-1 is associated with inflammation in the acute phase post-myocardial infarction (3, 4). Furthermore, *in vitro* and *in vivo* studies have provided evidence for the involvement of syndecan-1 in fibrosis and remodeling following angiotensin-II induced HF through the TGF-β/Smad-3 pathway. These studies demonstrated an increase of syndecan-1 expression in the heart following angiotensin-II infusion in which the ecto-domain of syndecan-1 plays a key role in the onset of fibrosis; blockade of the ecto-domain led to a diminished effect of angiotensin-II stimulation resulting in less collagen disposition (2). Shedding of the ecto-domain, leading to increased levels of soluble levels of the ecto-domain of syndecan-1 in plasma, may be part of a protective mechanism in HF. The correlation of shedding and detectable plasma levels is currently unknown. One may speculate that the loss of its ecto-domain might inhibit the function of the syndecan-1 receptor in activating the smad3/TGF-β pathway. Moreover, the soluble ecto-domain has been suggested to retain its binding properties, reducing the bioavailability of syndecan-1 receptor ligands (5).

The ecto-domain of the syndecan-1 protein has been shown to shed under the influence of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) (16). Previous

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**Table 4.** Risk stratification improvement of syndecan-1 levels on top of established clinical risk factors for both endpoints in patients with HFrEF and HFpEF

<table>
<thead>
<tr>
<th>Syndecan-1 (per doubling)</th>
<th>NRI*</th>
<th>p-value</th>
<th>IDI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HFrEF (n = 353)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined endpoint</td>
<td>0.026</td>
<td>0.816</td>
<td>0.001</td>
<td>0.674</td>
</tr>
<tr>
<td>3 year all-cause mortality</td>
<td>0.006</td>
<td>0.952</td>
<td>0.001</td>
<td>0.517</td>
</tr>
<tr>
<td><strong>HFpEF (n = 107)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined endpoint</td>
<td>0.485</td>
<td>0.016</td>
<td>0.031</td>
<td>0.026</td>
</tr>
<tr>
<td>3 year all-cause mortality</td>
<td>0.031</td>
<td>0.56</td>
<td>0.029</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*COACH risk engine model includes: age, sex, blood pressure, pulse pressure, history of stroke and/or MI, presence of atrial fibrillation, peripheral artery disease and/or diabetes, renal function, levels of NT-proBNP sodium, and previous HF hospitalization. Abbreviations: HFrEF, heart failure with reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; IDI, integrated discrimination improvement; NRI, net reclassification improvement.
studies have shown the subtle balance between MMPS and TIMPS to be primarily responsible for the cleavage of the syndecan-1 ecto-domain from the cell-surface; a balance that has readily been shown to be disturbed in HF, leading to measurable levels of the syndecan-1 ecto-domain in plasma (16–18). In addition, cellular syndecan-1 levels have been shown to be increased in WT mice in a HF model after angiotensin-II stimulation, the relative increase of shedding of the ecto-domain of syndecan-1 has however not been shown (2). To determine whether the shedding of the ecto-domain of syndecan-1 has a protective or harmful effect, additional evidence for the relative share of shedding of the syndecan-1 ecto-domain to the possible increased expression of syndecan-1 on a cellular level during HF is needed. Furthermore, HFP EF-induced fibrosis might be altered by directly or indirectly influencing the activity of the syndecan-1 receptor through syndecan-1 receptor antagonists or through decreasing the bioavailability of syndecan-1 and possibly increasing the presence of soluble syndecan-1 by influencing the tissue inhibitors of metalloproteinase/matrix metalloproteinase balance. However, more research has to be done to unravel the specific ligand(s) of syndecan-1 and how these relate to HF.

Interestingly, Cox regression analysis showed that syndecan-1 was related to clinical outcome in HFP EF patients, but not in HFrEF patients, which is independent of other known HF risk factors and the earlier reported correlation between sex and syndecan-1 levels (6). In addition, syndecan-1 showed prognostic value by adding it to known risk factors in HF as defined in the COACH risk model for the primary endpoint for HFP EF patients. Significant added value was not observed in NRI/IDI analysis for the secondary endpoint, however this could be explained by the nature of the COACH risk model, which is particularly designed for the primary endpoint in the COACH trial (13). This is of particular interest because syndecan-1 appears to be a marker for collagen turnover, which is suggested to play a central role in the pathophysiology of HFP EF (19). As such, this study shows that syndecan-1 has both prognostic value for the combined endpoint at 18 months as well as all-cause mortality at 3 years, this may indicate a possible biological involvement of syndecan-1 in the pathophysiological process of HFP EF on short- as well as long-term follow-up, suggesting an ongoing involvement of syndecan-1 throughout the progression of HFP EF. In addition, a significant interaction for syndecan-1, sex, and the secondary end point was found, as reported earlier (6). When dividing patients with HFP EF by sex, a significant predictive value was found for female patients but not for male patients. The results with regard to sex should, however, be critically interpreted because of the small size of the sex subgroups in the HFP EF population and the accompanying wide CIs, especially because no interaction was observed for syndecan-1, sex, and the primary end point. Additional research is needed to explore its role as a possible new marker in the treatment of patients with HFP EF. However, our observations are in line with a previous study published by our group, where we showed that the fibrotic biomarker galectin-3 has particular value in patients with HFP EF (11). Herein, we also found an interaction between the association of syndecan-1 and clinical outcome. This study provides further support for such an association between collagen and HFP EF, but less so for HFrEF.
Limitations

This is a post hoc analysis, warranting the possibility of a selection bias. Furthermore, the relatively small number of patients limits the prognostic value of syndecan-1 in HFpEF in this study. Sampling of patients in the COACH trial was performed at time of discharge, when patients were already recompensated. As such, this study includes patients who, at time of sampling, cover a gray area between acute and chronic HF. Furthermore, measurements of syndecan-1 were plagued by relatively high intra- and interassay coefficients of 25% and 25%, respectively, providing for possible variations between measurements. The findings reported in this study should not be regarded as providing evidence for a causal relationship, but should be seen in a more exploratory context. With regard to the role of syndecan-1 in patients with HFpEF, more research is needed in populations in which solely patients with HFpEF are included.

CONCLUSIONS

In patients with HF, syndecan-1 levels strongly correlate with other fibrosis markers pointing toward a role in cardiac fibrosis and remodeling. Syndecan-1 was independently associated with clinical outcome in patients with HFpEF but not in patients with HFrEF.
REFERENCES

CHAPTER 2

The fibrosis marker sybdecan-1 and outcome in heart failure patients with reduced and preserved ejection fraction

Jasper Tromp