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Endovascular Shedding Markers in Patients with Heart Failure with Reduced Ejection Fraction

Results from a single-center exploratory study

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Abstract

Background: Endothelial glycocalyx degradation has been associated with multiple pathophysiological processes in cardiovascular disease.

Aims: To explore the role of glycocalyx shedding markers in pathophysiology of heart failure with reduced ejection fraction (HFrEF).

Methods: In 123 HFrEF patients the concentration, prognostic value and association of glycocalyx shedding markers with other disease processes was investigated.

Results: Median hyaluronic acid (HA) levels and syndecan-1 levels in HFrEF patients were respectively 29.4(10.7;61.6) ng/ml and 48.5(33.6;80.8) ng/ml. Overall, HA-levels were significantly higher in HFrEF patients compared to healthy subjects but only 31% of HFrEF patients had HA levels above the cutoff of normal. There was no significant difference among HFrEF patients and healthy subjects regarding syndecan-1 levels. HFrEF patients with elevated HA- levels had a significantly worse outcome (log rank=0.01) which remained significant after correction for established risk factors (HR 2.53 (1.13-5.69); p=0.024). There was no significant relation between levels of shedding markers and neurohumoral activation (plasma renin activity, serum aldosterone, NT-proBNP), myocardial injury (HS-trop), inflammation (CRP) or other baseline characteristics.

Conclusion: The glycocalyx shedding marker HA is significantly elevated in a subgroup of HFrEF patients and an independent predictor for worse clinical outcome. Glycocalyx shedding might be an additional factor in the pathophysiology of HF which warrants further investigation.

Key words

Hyaluronic acid, Syndecan-1, Glycocalyx, Systolic Heart Failure, outcome

Abbreviations:

BUN: Blood urea nitrogen

CRP: C-reactive protein

eGFR: estimated Glomerular filtration rate

GAG: glycosaminoglycan

HA: hyaluronic acid

HF: Heart failure

HFREF: Heart failure with reduced ejection fraction

Na^+: sodium

NT-proBNP: N-terminal of the prohormone of B-type Natriuretic Peptide

NYHA: New York Heart Association

RAAS: renin-angiotensin-aldosterone system

Introduction

The endothelial glycoclayx is the inner fragile layer of the endothelium and composed of a network of membrane-bound and different types of glycosaminoglycans (GAG) and proteoglycans [1] (Figure 1A). The glycocalyx has multiple vasoprotective functions: it reduces vascular permeability, acts as a mechano-transducer of shear stress and prevents interaction

of platelets and leucocytes with endothelial cell adhesion molecules [2]. Moreover, recent evidence suggests that the endothelial GAG network acts as a sodium buffer by binding positively charged Na+ cations [3].

Various conditions can lead to disruption of this endothelial barrier such as ischemia and hypoxia [4-7], oxidative stress and inflammation [8-15], hyperglycemia [16], volume and salt overload [17], etc. It has already been demonstrated that a dysfunctional glycocalyx is involved in the process of atherosclerosis, endothelial dysfunction, tissue edema, renal dysfunction and related to increased cardiovascular events in different patient populations [5, 18-25]. Therefore, there is growing interest to further study glycocalyx integrity. However, in vitro and in vivo structural and/or functional assessment of the glycocalyx remains cumbersome [26-28]. Currently, the best way to investigate endothelial glycocalyx integrity is through the presence of glycocalyx shedding markers in plasma. Hyaluronic acid (HA) and syndecan-1 are the main constituents of the endothelial glycocalyx and elevated serum levels indicate degradation (figure 1) [29].

We recently hypothesized that increased glycocalyx shedding might also be present in heart failure (HF) and relate to prognosis [30]. The clinical value of shedding markers as a prognosticator in HFrEF has not been established yet. The objectives of this exploratory study are three-fold: 1) to study if levels of HA and syndecan-1 are increased in HFrEF patients or in specific subgroups reflecting glycocalyx shedding; 2) to investigate whether glycocalyx shedding is related to other processes present in HF; and 3) to study the prognostic value of these markers.

Methods

This study was carried out in a tertiary care center (Ziekenhuis Oost-Limburg, Genk, Belgium). The study complies with the Declaration of Helsinki and the institutional review board approved the study protocol.

Patient population

The current study cohort is the result of a pooled analysis of 3 investigator-initiated prospective studies in HFrEF patients with overlapping baseline and clinical outcome data, and availability of a venous blood sample which was obtained, processed and stored in similar conditions. All subjects were recruited in a single tertiary care center (Ziekenhuis Oost-Limburg, ZOL Genk) between January 2013 and May 2016. In each cohort, consecutive patients were included. Subjects were eligible for study inclusion if ≥18 years of age and able to give informed consent. All subjects had a prior diagnosis of heart failure with evidence of impaired left ventricular ejection fraction ≤40%. Exclusion criteria were: 1) renal replacement therapy or severe renal dysfunction with an estimated glomerular filtration rate ≤15 ml/min/1.73m^2 determined by the Chronic Kidney Disease Epidemiology Collaboration equation; [31] 2) administration of intravenous medication within 1 month of inclusion; 3) concurrent diagnosis of an acute coronary syndrome; or 4) concurrent diagnosis of an infectious or inflammatory disease.

Data collection

All subjects were screened by 2 heart failure specialist (W.M. and M.D.) at the outpatient clinic or emergency room of our center. After completion of informed consent, all patients underwent collection of detailed baseline characteristics including severity of HF (New York Heart Association (NYHA)-functional class), registration of comorbidities, baseline medication, clinical parameters and a clinical examination for signs of decompensation. Based on a clinical congestion score (table 1 supplemental material), patients were classified as stable heart failure patients if the clinical decongestion score was ≤1. In contrast, patients were classified as decompensated if having a clinical congestion score of >1 leading to a change in maintenance dose of loop diuretics and/or vasodilators or admission to the hospital.

Biochemical analysis

All blood samples were obtained after a period of at least 15 minutes in the semi-supine position and before admission to the hospital or administration of new medication. Samples were immediately processed and stored at -80°C until analysis was performed. Plasma N-terminal of the prohormone of B-type natriuretic peptide (NT-proBNP) levels were measured by the Roche Diagnostics Assay (Roche, Rotkreuz, Switzerland). Plasma renin activity (PRA) was determined using the Gamma-coat*radio immunoassay (DiaSorin, Sallugia, Italy). Plasma aldosterone levels were assessed by the Aldosterone Maia radioimmunoassay (Adaltis, Rome, Italy). Estimated glomerular filtration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [32]. Levels of hyaluronic acid were measured using a commercially available ELISA-kit (Cisbio Bioassays HYAL-US). Reported intra- and inter-assay coefficients of variation are <3% and <7% respectively. Levels of Syndecan-1 were measured using a commercially available ELISA-kit (Cusabio Human Syndecan-1). Reported intra- and inter-assays coefficient of variations (CV) are respectively <8 and <10% while measured intra-assay were 2.1% and 13.4%.

End point

The study end point was defined as the combined end point of all-cause mortality and heart failure readmissions (defined as hospitalizations due to signs or symptoms of congestion or low cardiac output that warranted treatment with parenteral drugs). Vital status and hospitalizations were retrieved from the hospital medical electronic records which is linked to the national death registry. From the day of inclusion, all events were prospectively registered up till 2 years.

Statistical analysis

Continuous variables are expressed as mean ± standard deviation if normally distributed, or otherwise as median [interquartile range]. Normality was assessed by the Shapiro-Wilk statistic. The unpaired student's t-test and Mann-Whitney test were used when appropriate. Categorical data were expressed as percentages and compared with the Pearson χ^2-test. Multiple linear regression models were constructed after a *2-log* transformation of HA and syndecan-1 to establish clinical determinants of *doubling* of shedding markers and its relation to other factors. Variables with a significant univariate association (<0,10) were entered in a stepwise forward multivariate model based on the strength of their univariate association. Unadjusted time-to-event comparisons between HFrEF patients with HA levels below or above the cutoff of normal were conducted using Kaplan-Meier survival estimates and log-rank test. For adjusted analyses, a Cox proportional hazards regression model was used to estimate hazard ratios with corresponding 95% confidence interval. The adjusted hazard ratios were corrected for established risk factors. Statistical significance was always set at a 2-tailed probability level of <0.05. All statistics were performed using SAS JMP Pro (version 11.2 for Windows).

Cut-off of normal value of shedding products

Normal values for HA and syndecan-1 were obtained in a cohort of 30 healthy subjects of which venous blood samples were simultaneously obtained, processed and analyzed with samples of HFrEF patients. Cut-off values were determined based on the *Robust method* as recommended by the Clinical and Laboratory Standards Institute [33, 34].

Results

Cut-off values of normal for HA and syndecan-1

Baseline characteristics of the cohort of healthy subjects (n=30, mean age 27±10) are presented in the supplemental material (Table 2 supplemental material). The median plasma level of HA was 18.9 (12.1;29.7) ng/ml and the median value for plasma syndecan-1 was 54.5 (32.2;130.3). The cut-off value of normal (the upper limit of normal) for HA was 50.2 ng/ml and 365.4 ng/ml for syndecan-1.

Characteristics of HFrEF patients

HFrEF patients were on average 66±13 years old with a mean left ventricular ejection fraction of 28±10%. Fourty four % of HFrEF patients were clinically stable while 56% were decompensated (Table 3 supplemental material). Baseline characteristics for all HFrEF patients are presented in Table 1.

Shedding markers in HFrEF patients

Median HA level in HFrEF patients was 29.4 (10.7;61.6) ng/ml. Overall, the cohort of HFrEF patients had significantly higher HA levels compared to normal subjects. Of this cohort, 31% had an elevated HA-level. Characteristics of HFrEF patients with versus without elevated HA-levels are compared in Table 2. The group of HFrEF patients with elevated HA-levels was significantly older (69±14 vs 64±11 years; p=0.044) and more were decompensated (71% vs 50%; p= 0.03). Although the difference in absolute values is small, plasma levels of C-reactive protein (CRP) were higher in this group (4.0(1.6;23.0) vs 3.0(1.2;6.3) mg/dl and maintenance therapy with angiotensin converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARB) was lower (52% vs 75%, p=0.014).

Furthermore, looking at decompensated HFrEF patients versus stable HFrEF patients, the former group had significantly higher levels (29.4 (11.6;92.2) vs 26.5 (37.2) ng/ml; p=0.024) while 20% of stable HFrEF patients had elevated HA-levels versus 39% of decompensated patients.

The median syndecan-1 levels in HFrEF patients was 48.5 (33.6;80.8) ng/ml. There was no significant difference between syndecan-1 levels of healthy subjects and HFrEF patients or between stable HFrEF patients and decompensated HFrEF patients (Figure 2). None of the HFrEF patients exceeded the cut-off value of normal for plasma levels of syndecan-1.

Association between shedding markers and other variables

To assess whether shedding markers were associated with different processes or variables in HFrEF such as neurohumoral activation (PRA and serum aldosterone), natriuretic peptide activation (NT-proBNP), inflammation (CRP), myocardial injury (HS-trop), renal dysfunction (blood urea nitrogen (BUN) and eGFR), and other baseline characteristics; a multivariable regression analysis was performed (supplemental material Table 4). There was a positive association found between doubling of HA (2-log transformation) and age in HFrEF patients. However, this correlation was poor (R^2=0.05, p=0.018) and was non-significant between absolute values of HA and age (R^2=0.03; p=0.070). No significant association could be observed between Syndecan-1 and any clinical variable tested.

HA and Clinical Outcome in HFrEF patients

During a mean follow up of 16±8 months a total of 40 events occurred of which 20 deaths and 20 HF -associated hospitalizations. Figure 3 illustrates Kaplan-Meier curves for the combined endpoint in HFrEF patients with normal versus elevated HA-levels

(characteristics of both groups are presented in Table 2). Elevated HA levels showed a significant increase for the combined end point after adjusting for baseline differences (age, CRP, ACE-inhibitor/ARB use, decompensated state) (hazard ratio (HR) 2.76; 95% confidence interval (CI) 1.68-6.79; p=0.021) or established outcome related variables (age>75years, medical history of myocardial infarction, NT-proBNP level and renal function<60 ml/min/1.73m2) (hazard ratio (HR) 3.32; 95% confidence interval (CI) 1.36-8.18; p=0.009)(Table 3). There was no significant relation found between syndecan-1 and outcome in HFrEF patients.

Discussion

This study aimed to explore the presence and prognostic value of glycocalyx shedding markers in HFrEF. Our main observations are that 1) elevated HA-levels are present in 31% of HFrEF patients in our cohort. There was no difference in syndecan-1 levels between healthy subjects and HFrEF patients 2) increased HA is a specific and independent predictor for clinical outcome in patients with HFREF. 3) No significant relationship between shedding markers or any measured parameter related to HF could be established in our pilot study. Therefore, we hypothesize that glycocalyx shedding might be an additional factor in the pathophysiology of HF which warrants further investigation.

Shedding products in HFrEF patients and associate factors

The major constituents of the glycocalyx are hyaluronic acid (or hyaluronan), and syndecan-1 (Figure 1) [35]. Data on HA levels in HF are extremely sparse. One small Chinese study observed higher levels of HA in higher NYHA classes of congestive HF compared to NYHA-class 1 [36]. We found that levels of HA are significantly higher in patients with HF compared to healthy controls. However, of the total cohort, only 31% of patients had elevated

HA-levels. We compared HFrEF patients with versus without elevated HA-levels and found that there was a significant difference regarding age, presence of decompensation, CRP-level and maintenance therapy with ACE/ARB. Indeed, inflammation or infection, oxidative stress, volume and salt overload, but also ischemia and hypoxia are conditions which are known to be associated with glycocalyx shedding. These processes are frequently present in HFrEF patients, especially during decompensation. This might explain why more patients with clinical signs of decompensation had elevated HA-levels. We observed elevated HA-levels in 39% of decompensated patients versus 20% of stable HFrEF patients ($p<0.05$). Similarly, previous experiments demonstrated that activation of the natriuretic peptide system induces glycocalyx shedding [37, 38]. HFrEF patients with higher levels of HA took significantly less ACE-inhibitors/ARB as maintenance therapy. Although not significant, these patients also took less mineralocorticoid receptor antagonists (MRA). It has been demonstrated that blockers of the renin angiotensin aldosterone-system, such as spironolactone, can protect and stabilize the glycocalyx [39]. However, not all patients with decompensation had evidence of glycocalyx shedding and 1 in 5 patients with a clinical stable status of heart failure had elevated levels of HA.

After multivariate regression analysis which included all these factors, the only independent factor associated with HA was age. Although the sample size of our cohort study is limited and therefore results should be interpreted with caution, this could indicate that the presence of glycocalyx shedding is an additional detrimental factor in the pathophysiology of heart failure. Most likely, glycocalyx shedding itself is multifactorial. The presence of shedding products in the plasma seem to indicate the presence of a different (independent) process aside from the classical neurohumoral, inflammation or injury pathways with a negative impact on prognosis.

We found a poor relationship between age and plasma HA-levels. Similar findings are published before [40, 41]. The normal aging process results in structural as well as compositional changes of extracellular matrix (such as the glycocalyx but also other

connective tissues in the body). This is the consequence of a disruption in the balance between synthesis and catabolism, which might also play a role in vascular stiffening [39]. However, the association between HA-levels and age in HFrEF patients in our study was weak.

We could not find a significant difference in levels of syndecan-1 between stable HFrEF and decompensated HF or even with healthy subjects. To our knowledge there are no studies comparing levels of syndecan-1 in patients with stable versus decompensated HFrEF or HFrEF and a normal control population. We found a wide distribution of syndecan-1 in healthy subjects (54.5 (32.2;130.3) ng/ml) which was more profound than found in other groups of healthy controls, and is likely responsible for the lack in significant differences or a meaning-full cut-off value [4, 42]. Moreover, but further discussed below, no association between syndecan-1 levels and outcome could be found.

Biomarker for glycocalyx shedding

HA may be a better biomarker for glycocalyx shedding than syndecan-1 for several reasons. HA is a long polymer of hundreds of disaccharide units that carry strong negative charges. However, in contrast to other GAGs, HA is not bound to a core protein. Most of the HA in the vasculature is incorporated in the endothelial glycocalyx [43]. Tissue half-life of HA can range between 0.5 and 3 days [44]. In contrast, syndecan-1 is a proteoglycan which is composed of a core protein with covalently bound GAG-chains. There are many cell-matrix interactions between syndecan-1 and surrounding structures. Its half-life is estimated around 6 hours due to rapid metabolization [45] (Figure 1).

Outcome

After adjusting for established HF risk factors, including age, in a multivariate cox model, renal dysfunction (eGFR<60 ml/min/1.73m2) and elevated levels of HA remained the only significant factors associated with outcome. These exploratory data indicate that further research in the area of glycocalyx shedding in HFrEF patients is more than justifiable.

Previous data on the prognostic value of syndecan-1 in HF patients is conflicting. Patients with acute decompensated HF and higher levels of syndecan-1 (>125 ng/ml) have a higher risk of 6-month mortality and renal dysfunction during hospitalization [23]. Another study of 2033 patients with acute HF after being stabilized during hospital stay evaluated the diagnostic accuracy of 44 biomarkers including syndecan-1 for heart failure. Syndecan-1 was significantly correlated with 30-day and 180-day mortality [46]. However, in other studies the relationship between syndecan-1 and mortality was only present in patients with HF and preserved ejection fraction but not in HFrEF patients [47]. We found no relation between syndecan-1 levels and outcome in HFrEF patients.

Future perspectives

Degradation of the endothelial glycocalyx is currently being associated with a growing number of cardiovascular diseases. Investigating the role of the endothelial glycocalyx in HF is promising due to its pathophysiological role in microvascular and endothelial function. The prevalence of HF is increasing and almost no new treatment strategies are discovered in the last two decades. Clinical studies indicating that protection of the endothelial glycocalyx from degradation benefits clinical outcome are lacking. Pharmacological agents such as inhibitors of inflammation, antithrombin, inhibitors of metalloproteases but also albumin and sulodexide, a preparation delivering precursors of glycocalyx constituents, display the potential to attenuate shedding of the glycocalyx. Analyses of HA and syndecan-1 levels in randomized controlled trials directed at medical therapy or other biomarkers in HFrEF but

also HF with preserved ejection fraction can help elucidate the role of glycocalyx shedding in the pathophysiology and prognosis of HF.

Study limitations

Although the strong statistical difference in HA and its prognostic relation, this was a small and single-center trial with inherent obvious limitations. Our findings should be considered hypothesis-generating and interpret as an exploratory study for further research in this domain. Due to the lack of a standardized in vivo investigation technique, glycocalyx structure and function was only indirectly studied by plasma-levels of shedding products. Additionally, the healthy control group which was used to derive normal values was much younger than heart failure patients. An age-matched control group could have given addition insights regarding normal values of plasma shedding products. Future research should take this into account.

Conclusion

This single-center exploratory study demonstrates that the glycocalyx shedding marker HA is significantly higher in HFrEF patients compared to healthy subjects and elevated in a subset of HFrEF patients. No significant difference could be found between HFrEF patients and healthy subjects for syndecan-1. Levels of shedding markers were not associated with neurohumoral activation, renal function or other variables. Elevated HA level is a specific and independent predictor for clinical outcome in patients with HFREF. Further trials regarding the role and prognostic importance of the endothelial glycocalyx are warranted.

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Conflict of interest

None

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Figure 1: The endothelial glycocalyx

Figure 2: Plasma values of shedding products in Healthy subjects and HFrEF patients.

Figure 3: Kaplan-Meier curves for combined endpoint in HFrEF patients stratified by HA value below versus above the cut-off of normal

Table 1: Baseline Characteristics of HFrEF patients

	HFrEF patients
n	123
age	66±13
male gender	84%
ischemic etiology	67%
BMI	29±5
LVEF%	28±10
Decompensation	
no	44%(n=54)
yes	56% (=69)
NYHA class	
I-II	46%
III-IV	54%
Systolic BP	125±21
Medical history	
- Myocardial infarction	67%
- Hypertension	41%
- Atrial fibrillation	38%
- Diabetes Mellitus	29%

Labarotory values	
- Hb (g/dl)	13.1±1.8
- Sodium (mmol/L)	138±3
- CRP (mg/dl)	3.1(1.2;8.4)
- Creatinine (mg/dl)	1.5±0.7
- BUN (mg/dl)	65±37
- eGFR (ml/min/1.73m2)	57±24
- NTproBNP (ng/L)	2345(951;6263)
- PRA (ug/Lh)	3.9(1.1;12.7)
- Serum aldosterone (ng/L)	193(139;358)
- HS-trop (ng/L)	22(12;40)
Plasma levels of shedding products	
- Syndecan (ng/ml)	48.5(33.6;80.8)
>cutoff	0%
- Hyaluronic Acid (ng/ml)	29.4(10.7;61.6)
>cutoff	31%
Maintenance therapy at inclusion	
- ACE/ARB use	68%
- BB use	85%
- MRA use	67%
- Loop diuretic use	59%
- Hydralazine/Nitrate use	21%

ACE: angiotensin converting enzyme inhibitor;ARB: angiotensin receptor blocker, BMI: body mass index; BP: blood pressure; BUN: blood urea nitrogen; COPD: chornic obstructive pulmonary disease; CRP: C-reactive protein; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonist; NYHA: New york Heart association-class; NT-proBNP: N-terminal of the pro-hormone of brain natriuretic peptide; PRA: plasma renin activity.

Table 2: Characteristics of HFrEF patients with normal levels of HA (< 50.2 ng/ml) or elevated levels

	HFrEF with normal HA-levels (<50.2 ng/ml)	HFrEF with Elevated HA-levels (>50.2 ng/ml)	p-value
n	85	38	
age	64±11	69±14	**0.044**
male gender	81%	89%	0.249
ischemic etiology	67%	66%	0.442
BMI	29±5	28±5	0.438
LVEF	29±10	27±10	0.266
Decompensation			**0.003**
No	50%	29%	
yes	50%	71%	
NYHA class	54%	29%	0.137
I-II	46%	71%	
III-IV	126±21	125±22	
Systolic BP			0.850
Medical history	67%	68%	
- Myocardial infarction	47%	29%	0.882
- Hypertension	38%	38%	0.060
- Atrial fibrillation	27%	34%	0.984
- Diabetes Mellitus			0.421
Labarotory values			
- Hemoglobin (g/dl)	13.1±1.7	12.9±1.9	0.376
- Na (mmol/L)	138±3	138.3	0.845
- CRP (mg/L)	3.0(1.2;6.3)	4.0(1.6;23.0)	**0.009**
- HS-Trop (ng/L)	19(10;35)	32(20;60)	0.779
- BUN (mg/dl)	65±34	66±43	0.885
- eGFR (ml/min/1.73m2)			
- NTproBNP (ng/L)			
- PRA (ug/Lh)			

-	Serum aldosterone (ng/L)	58.0±24.0	55.6±25.1	0.614
		1911(605;4515)	3558(1417;7535)	0.240
		4.5(1.3;13.9)	3.1(0.9;9.5)	0.060
		194(143;428)	176(133;272)	0.128
Plasma levels of shedding products				
-	Syndecan (ng/ml)	47.8(29.6;81.0)	49.7(38;73.5)	0.387
-	Hyaluronic Acid (ng/ml)	12.5(9.1;31.7)	95.4(95.5;67.8)	<0.001
Maintenance therapy at inclusion				
-	ACE/ARB use	75%	52%	**0.014**
-	BB use	87%	79%	0.250
-	MRA use	72%	59%	0.129
-	Loop diuretic use	62%	52%	0.311
-	Hydralazine/Nitrate use	21%	20%	0.910

ACE: angiotensin converting enzyme inhibitor;ARB: angiotensin receptor blocker, BMI: body mass index; BP: blood pressure; BUN: blood urea nitrogen; COPD: chornic obstructive pulmonary disease; CRP: C-reactive protein; LVEF: left ventricular ejection fraction; MRA: mineralocorticoid receptor antagonist; NYHA: New york Heart association-class; NT-proBNP: N-terminal of the pro-hormone of brain natriuretic peptide; PRA: plasma renin activity.

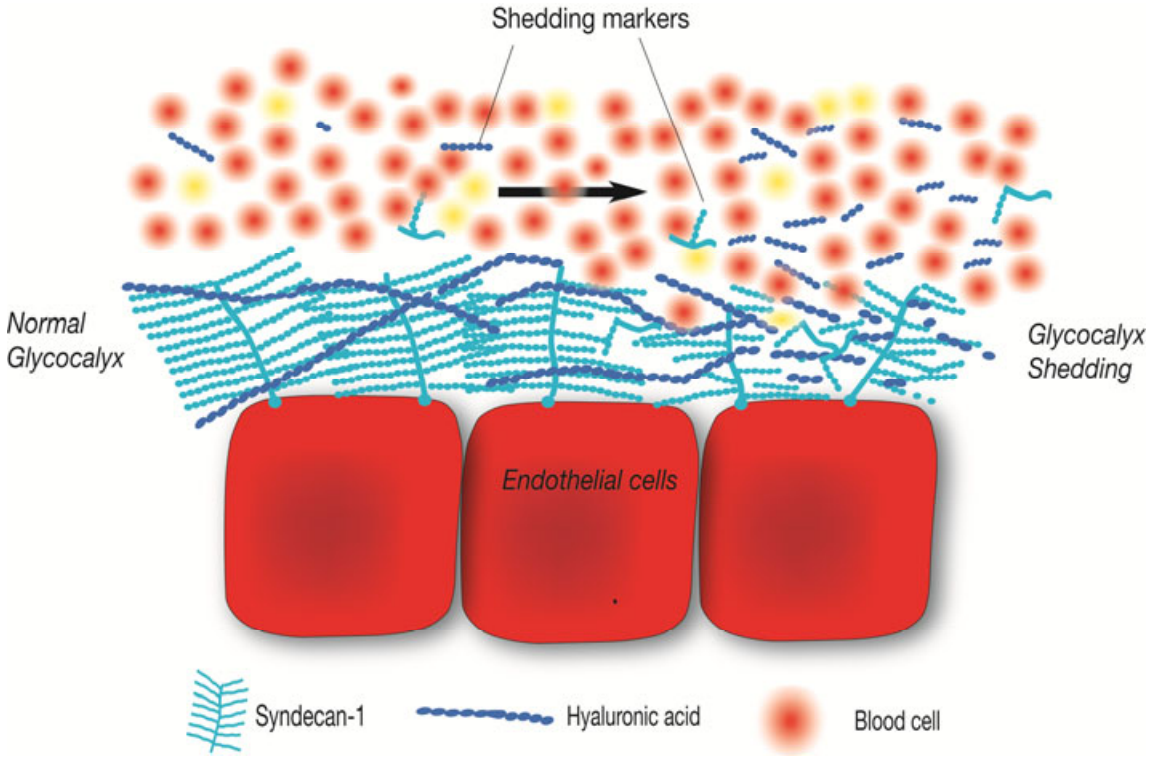
Table 3: Unadjusted and Adjusted hazard ratio for elevated HA levels regarding the combined endpoint of all-cause mortality and HF associated hospitalization.

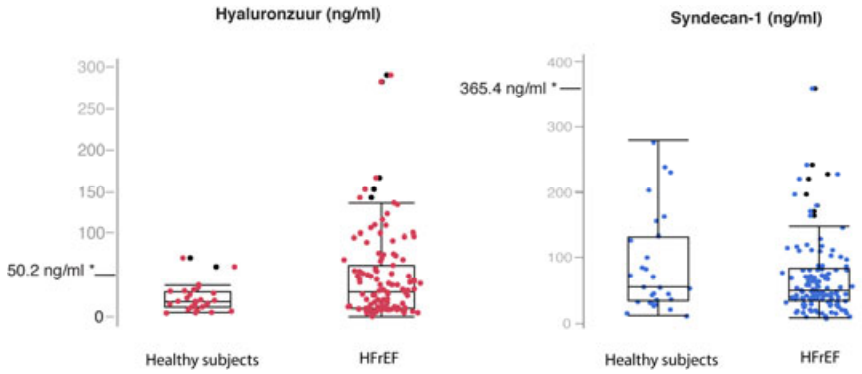
Unadjusted Hazard ratio			
Variable	**HR**	**95% CI**	**p-value**
Elevated HA	2.63	1.23-5.68	0.013

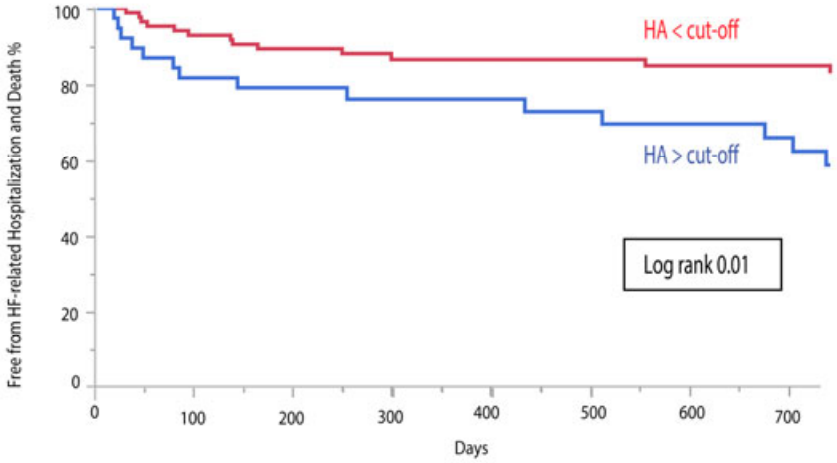
Adjusted Hazard ratio (1)			
Variable	**HR**	**95% CI**	**p-value**
Elevated HA	2.76	1.68-6.79	**0.021**
No ACE-inhibitor/ARB use	1.67	0.69-4.12	0.252
Decompensated	1.55	0.60-4.51	0.374
CRP (mg/dl)	0.97	0.93-1.00	0.202
Age> 75 years	1.47	0.60-3.49	0.385

Adjusted Hazard ratio (2)			
Variable	**HR**	**95% CI**	**p-value**
eGFR<60 ml/min/1.73m2	4.41	1.68-12.96	**0.002**
Elevated HA	2.52	1.13-5.69	**0.024**
History of myocardial infarction	2.53	0.95-8.78	0.065
NT-proBNP (ng/L)	1.21	0.17-6.47	0.832
Age>75 years	1.09	0.46-2.56	0.836

95%CI: 95% confidence interval, eGFR: estimated glomerular filtration function; HA: hyaluronic acid (elevated = >52.2 ng/ml), HR: Hazard ratio; NT-proBNP: N-terminal of the pro hormone of brain natriuretic peptide (ng/L). Hazard ratio (1) was adjusted for variables which significantly differed between HFrEF patients with elevated versus normal HA-levels. Hazard ratio(2) was adjusted for established risk factors in HF.







Nr at risk	Baseline	1 month	6 months	12 months	18 months	24 months
Red=<cutoff	85	84	76	57	54	43
Blue>cutoff	38	35	30	23	20	17