

# Left atrial rather than left ventricular impaired mechanics are associated with the pro-fibrotic ST2 marker and outcomes in heart failure with preserved ejection fraction

■ A. I. Nagy<sup>1</sup> , C. Hage<sup>2,3</sup>, B. Merkely<sup>1</sup>, E. Donal<sup>4</sup>, J.-C. Daubert<sup>4</sup>, C. Linde<sup>2,3</sup>, L. H. Lund<sup>2,3</sup> & A. Manouras<sup>2,3</sup>

From the <sup>1</sup>Heart and Vascular Center, Semmelweis University, Budapest, Hungary; <sup>2</sup>Theme of Heart and Vessels, Karolinska University Hospital; <sup>3</sup>Institution for Medicine, Karolinska Institutet, Stockholm, Sweden; and <sup>4</sup>Département de Cardiologie & CIC-IT U 804, Centre Hospitalier Universitaire de Rennes, Rennes, France

**Abstract.** Nagy AI, Hage C, Merkely B, Donal E, Daubert J-C, Linde C, Lund LH, Manouras A (Semmelweis University, Budapest, Hungary; Karolinska University Hospital; Karolinska Institutet, Stockholm, Sweden; Centre Hospitalier Universitaire de Rennes, France). Left atrial rather than left ventricular impaired mechanics are associated with the pro-fibrotic ST2 marker and outcomes in heart failure with preserved ejection fraction. *J Intern Med* 2018; **283**: 380–391.

**Aims.** Left ventricular (LV) mechanics have been extensively investigated in heart failure with preserved ejection fraction (HFpEF) overshadowing for a long time the potential role of left atrium (LA) in that setting. Soluble suppression of tumorigenicity-2 receptor (ST2) is a novel biomarker of pro-fibrotic burden in HF. We hypothesized that due to the thinner LA wall, the fibrotic myocardial changes in HFpEF as indicated by elevated ST2 levels might more readily be reflected by impairments in the LA rather than the LV performance.

**Methods and Results.** In 86 patients with HFpEF, enrolled in the Karolinska Rennes (KaRen)

biomarker prospective substudy, global LA strain (GL-LS) along with other echocardiographic as well as haemodynamic parameters and ST2 levels were measured. ST2 levels were inversely associated with LA-GS ( $r = -0.30$ ,  $P = 0.009$ ), but not with LA size, LV geometry, systolic or diastolic LV function ( $P > 0.05$  for all). Furthermore, symptom severity correlated with ST2 and LA-GS, but not with LV structural or functional indices. Finally, during a median 18-month follow-up, LA-GS independently predicted the composite endpoint of HF hospitalization and all-cause mortality, even after adjustment for potential clinical and cardiac mechanical confounders, including LV global longitudinal strain and filling pressures (odds ratio: 4.15; confidence interval: 1.2–14,  $P = 0.023$ ).

**Conclusions.** Reduced LA-GS but not LV functional systolic and diastolic parameters were associated with the pro-fibrotic ST2 marker, HF symptoms and outcome in HFpEF.

**Keywords:** ST2, heart failure, preserved ejection fraction, left atrial strain, prognosis.

## Introduction

Heart failure with preserved ejection fraction (HFpEF) accounts for up to half of patients with heart failure (HF) [1, 2], with a prognosis comparable to that of HF with reduced ejection fraction (EF) [1] and an increasing prevalence.

For a long time, investigations aiming to uncover the pathophysiology underlying HFpEF have focused on left ventricular (LV) mechanics;

however, no pathognomonic LV functional or structural alterations have been identified. While LV hypertrophy is common in HFpEF, nearly half of the HFpEF patients show no signs of hypertrophy [2]. Similarly, although increased LV stiffness is typical in this patient population [3, 4], it may also occur in subjects without clinical signs and symptoms of HF [5]. Furthermore, whereas LV diastolic dysfunction is considered as a hallmark of HFpEF, abnormal diastolic LV performance is an almost universal finding in elderly patients without HFpEF [5], which underscores the significance of other mechanisms in the pathophysiology of HFpEF.

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Recently, a subtle shift in the focus of interest in the HFpEF field has occurred with an increasing number of studies investigating the role of the left atrium (LA) rather than that of the LV in the HFpEF [6–9]. Elevated filling pressures and subsequent LA dilation are a characteristic finding in HFpEF. The degree LA enlargement reflects disease chronicity [10] and is prognostic in HFpEF [9]. Importantly, apart from the structural remodelling, increased LA stiffness has also been demonstrated in HFpEF, which further aggravates the elevated LA pressures, particularly during exertion.

Increasing evidence supports the concept that HFpEF ultimately develops on the basis of a pro-inflammatory state triggered by comorbidities [11]. The soluble suppression of tumorigenicity-2 receptor (ST2) is an established biomarker of inflammation and fibrosis with an emerging role in the diagnosis and prognostication of HF. In the context of HF, ST2 expression is thought to be triggered by myocardial stretch and consequent fibrosis [12]. In patients with reduced EF, ST2 has superior prognostic power compared to conventional biomarkers such as N-terminal pro-brain natriuretic peptide (NT-proBNP) [13]. Patients with HFpEF also demonstrate elevated ST2 levels. Interestingly however, in this condition no significant correlation between ST2 levels and LV structure or function has been found [14].

As HFpEF is postulated to develop as a result of a systemic inflammatory reaction, we hypothesized that the LA, given its much thinner wall, is likely to be more susceptible to fibrotic changes as compared to the LV. Thus, early signs of the disease, as indicated by elevated ST2 levels, might more promptly manifest in altered LA mechanics, rather than in impaired LV performance. Accordingly, employing information from the biomarker Karolinska Rennes (KaRen) prospective, multicentre substudy, we tested the hypothesis that in HFpEF (1) plasma ST2 levels correlate with LA function; and (2) impaired LA function is associated with worse outcomes.

## Materials and methods

### Study population

The study included 86 HFpEF patients enrolled in the prespecified KaRen Biomarker Study which comprised a substudy of the Karolinska Rennes (KaRen) prospective, observational, multicentre study [15]. Patients admitted for acute HF

symptoms, NT-proBNP  $>300 \text{ ng L}^{-1}$  and LVEF  $\geq 45\%$  were enrolled between May 2007 and December 2011. Four to 8 weeks after enrolment, when patients were in a stable condition, blood samples were taken and clinical investigations, including echocardiography, were performed. The patients were followed until September 2012 when vital status was assessed by telephone contact or by the Swedish National Patient and Population Registers and then centrally adjudicated. All HF hospitalizations were adjudicated and defined according to clinical judgement by the local specialist investigator and additionally centrally validated to confirm the presence of HF at hospitalization. The primary outcome was the composite of time to death from any cause or first hospitalization for HF (Figure S1).

### Biomarker assays

Fasting blood samples were taken from subjects in a stable condition and euvoaemic state, collected in chilled EDTA tubes, immediately centrifuged at  $4^\circ\text{C}$  and stored in aliquots at  $-70^\circ\text{C}$  until analysis. NT-proBNP was analysed by proBNP II (Roche Diagnostics, Bromma, Sweden). Plasma ST2 levels were measured by the Presage ST2 Assay (Critical Diagnostics, San Diego, California). Estimated glomerular filtration rate (eGFR) was calculated according to the MDRD study equation:

$$\text{GFR [mL/min/1.73 m}^2\text{]} = 175 \times [\text{creatinine}]^{-1.154} \\ \times [\text{Age}]^{-0.203} \times [0.742 \text{ if female}]; \\ \text{Creatinine in mg/dL, age in years.}$$

### Echocardiographic data

All subjects underwent transthoracic echocardiography using a Vivid-7 system (GE Ultrasound, Horten, Norway) equipped with a 2.5-MHz matrix array transducer. Images were analysed offline (EchoPAC PC, version 2.0 GE Ultrasound, Waukesha, Wisconsin) by a single echocardiographer, blinded to the patients' clinical data. Stroke volume index (SV<sub>i</sub>) was measured by Doppler method. For LA volumetric analysis, the method of disc method was used. Myocardial deformation was analysed by two-dimensional speckle tracking, using dedicated software designed for the LV and LA, respectively (TomTec Imaging Systems, Unterschleissheim, Germany). LV global longitudinal strain (LV-GLS) was calculated as the average of longitudinal strain measured in 12

segments obtained from the apical four- and two-chamber views. LA global strain (LA-GS) was measured in the apical two-chamber view, according to the manufacturer's recommendations. The LA endocardial border was traced so that the LA appendage and pulmonary veins were excluded. LA reservoir function was estimated by peak LA-GS during ventricular systole. All measurements were averaged over 3 cardiac cycles. At the time of echocardiographic examination, 21 patients were in atrial fibrillation (AF). In these cases, measurements were averaged over 5 cycles. In case of significant foreshortening of the cavity or poor tracking quality, the measurements were considered unreliable and excluded from the analysis ( $n = 7$ ).

Intra-observer variability for LA-GS measurements was assessed in 10 randomly selected patients. The coefficient of variation was 8%, and the intraclass correlation coefficient was 0.94 (95% CI = 0.631–0.991).

#### *Measurements of the atrioventricular coupling and vascular function*

Effective arterial elastance ( $E_a$ ) constitutes a 'lumped index' of LV afterload in the time domain and was calculated as  $E_a = \text{LVESP}/\text{SV}$ , where LVESP is the LV end-systolic pressure. LVESP values were estimated as derived from the equation:  $\text{LVESP} = 0.9 \times \text{SBP}$ , where SBP is the systolic systemic blood pressure [16]. LV end-systolic elastance ( $E_{es}$ ) was calculated using the single-beat approach developed by Chen *et al* [17]. Total arterial compliance was estimated by the SV-to-pulse pressure ratio [18] and systemic vascular resistance index (SVRI) as: mean arterial pressure/cardiac index  $\times 80$ .

#### *Assessment of LV relaxation rate and filling pressures*

The mean value of the lateral and septal mitral annular early diastolic velocity ( $e'$ ) was determined by spectral tissue Doppler imaging using standard methods. The  $e'$  velocity is relatively preload independent and inversely related to the time constant of isovolumic relaxation ( $\tau$ ), which was derived from the previously validated formula:

$\tau = (14.70 - 100 \times e')/0.15$  [19]. Early transmitral flow velocity ( $E$ ) was measured by pulsed-wave Doppler. LV end-diastolic pressure (LVEDP) was estimated as follows:  $\text{LVEDP} = 11.96 + 0.596 \times E/e'$ ,

as previously determined from Doppler and invasive EDP measurements [19].

#### *Determination of LV diastolic stiffness*

The validated single-beat approach [20] was used to characterize the LVEDP–end-diastolic volume (EDV) relationship (EDPVR) based on the equation:  $\text{EDP} = \alpha \times \text{EDV}^\beta$ ; where  $\alpha$  is a curve-fitting constant and  $\beta$  is the diastolic stiffness constant describing the steepness of the EDPVR curve. Measured EDP and EDV were used to derive  $\alpha$  and  $\beta$  in each subject. Additionally, LV end-diastolic stiffness was assessed by the ratio between EDP and EDV.

#### *Ethics*

The study conformed to the Declaration of Helsinki had ethics approval by local ethics committees, and all participants provided written informed consent.

#### *Statistics*

IBM SPSS statistics version 23.0 (IBM Corp., Armonk, NY, USA) was used. Normality was tested using the Kolmogorov–Smirnov test. Continuous variables were expressed as mean  $\pm$  SD or median and interquartile range (IQR) whereas categorical variables as absolute values and percentage.

Comparisons between groups were performed with Mann–Whitney rank-sum test. Correlations were tested by the Pearson's two-tailed test. All tests were performed at 95% confidence intervals. All  $P$ -values were two-sided, and statistical significance was, except for the Bonferroni-adjusted correlations, set at 0.05. HFpEF patients were categorized according to quartiles of LA-GS, and trend tests were applied across the groups to investigate the association between LA-GS and demographic characteristics and echocardiographic measures of cardiac structure and function.

The association of LA-GS with the combined outcome of death and/or hospitalization was tested with univariate and multivariable Cox proportional hazards models and Kaplan and Meier nonparametric test and compared using a log-rank test, using a time-to-event analysis. Adjustment for demographic and clinical covariates (age, history of AF, logST2, eGFR, LV-GLS, LAVi,  $E/e'$  and  $E_a$ ) was performed. The proportional hazards assumption was tested for

all analyses. No violation of the proportional hazards assumption by LA-GS was found.

Because of the biomarker levels not being normally distributed, all biomarker data were natural-logarithmically (log)-transformed.

Analysis of inter- and intra-observer variability was performed for LA-GS in 10 randomly selected patients by two observers. Methodological error (Err) in a single measurement estimated from double measurements was calculated according to formula:  $\text{Err} = (\text{SD}_{\text{diff}} \times 100\%) / (\text{total mean} \times \sqrt{2})$ , where  $\text{SD}_{\text{diff}}$  is the SD of the difference between the measurements [21].

## Results

Demographic characteristics of the patient population are provided in Table 1. Median EF for the whole cohort was 63% (Q1:57%, Q3:68%). At the time of enrolment, all patients were highly symptomatic (88% in New York Heart Association functional class (NYHA) III-IV, 12% in NYHA II status); however, when the echocardiographic examination and biochemical analyses were performed (in stable state 4–8 weeks after enrolment), the symptoms were significantly alleviated (17% in NYHA III, 59% in NYHA II and 23% in NYHA I; 69% on diuretics).

Similar to prior studies in HFpEF, there was a slight over-representation of women (51% vs. 49%) and nearly half (41%) of the patients demonstrated severe obesity. Fifty-two patients (60%) had previously been diagnosed with AF, of whom 21 were in AF at the time of the echocardiographic examination.

### ST2 levels and cardiac mechanics

The median ST2 concentration in our cohort was  $32 \text{ ng mL}^{-1}$  [Q1:24–Q3:48  $\text{ng mL}^{-1}$ ]. Serum ST2 levels inversely correlated with LA-GS ( $r = -0.30$ ,  $P = 0.009$ , Fig. 1a). However, no association was found between ST2 and the degree of LA enlargement (LA volume index, LAVi) or with indices of LV geometrical remodelling (LV mass index (LVMI), LV systolic and diastolic volumes), LV systolic functional parameters (LVEF, LV-GLS,  $E_{\text{es}}$ ), measures of the LV relaxation and end-diastolic function ( $\tau$ ,  $\beta$ , EDP/EDV,  $E/e'$ ) or indices of the AV-coupling and the systemic vascular function ( $E_{\text{a}}/E_{\text{es}}$ , arterial compliance, SVRi). Importantly,

**Table 1** Demographic data

General	
Age years	72 ± 10
Gender male/female	42/44 (49/51)
Medical history	
Atrial fibrillation/flutter	52 (60)
Hypertension	68 (79)
Diabetes mellitus	28 (33)
COPD	17 (20)
Cancer	15 (17)
Coronary disease	13 (15)
NYHA I	19 (22)
NYHA II	46 (53)
NYHA III	19 (22)
NYHA IV	0
Clinical measurements	
BMI $\text{kg m}^{-2}$	30 ± 6
Obesity (BMI ≥ 30)	34 (41)
SBP (mmHg)	142 ± 21
DBP (mmHg)	79 ± 9
HR (beats/min)	70 ± 15
Treatment	
ARB or ACE-I	65 (76)
Statin	37 (43)
Digoxin	10 (12)
Loop diuretic	59 (69)
Beta-blocker	67 (80)
Calcium channel blocker	26 (30)
Laboratory findings	
NT-proBNP ( $\text{ng}\cdot\text{L}^{-1}$ )	1000 (Q1:465;Q3:2335)
ST2 ( $\text{ng}\cdot\text{mL}^{-1}$ )	32 (Q1:24;Q3:48)
eGFR ( $\text{mL}\cdot\text{min}^{-1}/1.73 \text{ m}^2$ )	70 (Q1:54;Q3:85)
Haemoglobin ( $\text{g}\cdot\text{L}^{-1}$ )	13.1 (Q1:12.2;Q3:14.2)
White blood cell count (10 <sup>9</sup> /L)	8.0 (Q1:7.1;Q3:9.9)

COPD, chronic obstructive pulmonary disease; NYHA, New York Heart Association functional class; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; ARB, angiotensin receptor blocker; ACE-I, ACE inhibitor; NT-proBNP, N-terminal pro-brain natriuretic peptide; ST2, soluble suppression of tumorigenicity-2 receptor; eGFR, estimated glomerular filtration rate. Data are provided as absolute numbers followed by percentages in brackets; or median values followed by 1st (Q1) and 3rd (Q3) quartiles in brackets

an inverse association of ST2 with RV function, as assessed by tricuspid annular plane systolic excursion (TAPSE), was demonstrated ( $r = -0.28$ ,  $P = 0.01$ ), whereas no correlation between ST2 and renal functional indices (s-Creatinine, eGFR) or CRP was observed.

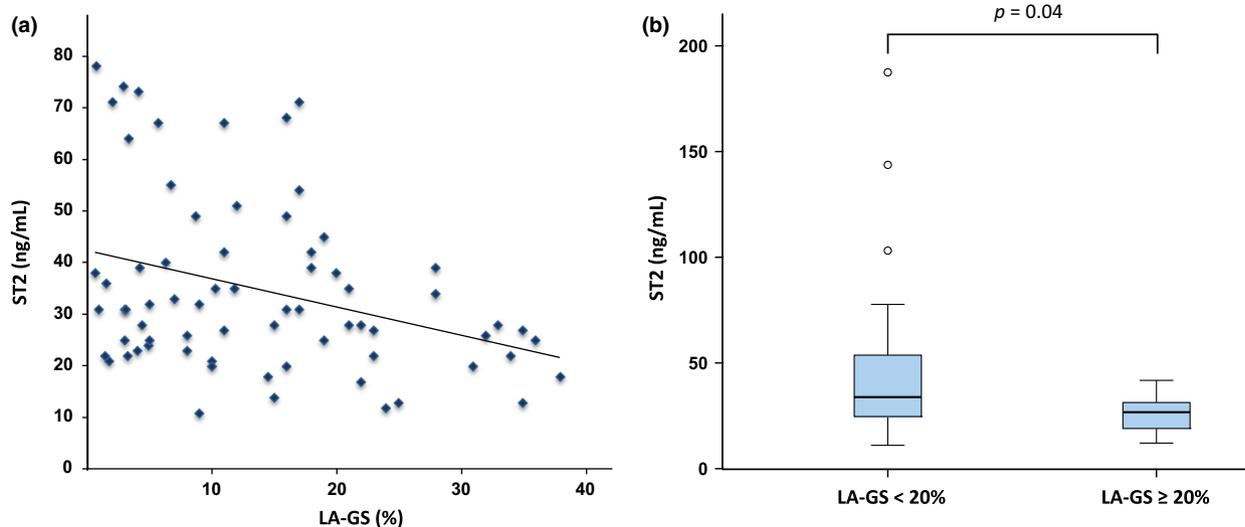
#### Determinants of LA strain

The LA-GS did not show significant association with the LVMI or LV volumes. Although it was significantly related to the SV ( $r = 0.23$ ,  $P < 0.05$ ), no correlation with other indices of LV systolic performance (LVEF, LV-GLS;  $E_{es}$ ) was evident. Similarly, LA-GS was not associated with either the LV end-diastolic function as assessed by EDP/EDV ratio, or the  $\beta$  value representing the slope of the EDPVR or the preload as estimated by  $E/e'$ . On the other hand, LA-GS was inversely related with LV afterload as described by  $E_a$  ( $r = -0.28$ ,  $P = 0.01$ ). Importantly, no significant relationship between LA-GS and LAVi was found. Similar to ST2, LA-GS was also significantly associated with TAPSE ( $r = 0.41$ ,  $P < 0.001$ ). In a multiple regression analysis, including logST2, age, eGFR, LV-GLS, LAVi,  $E/e'$  and  $E_a$  as potential predictors, only logST2 and eGFR were identified as independent predictors of the LA-GS (LA-GS =  $39.7 - 5.4 * \logST2 - 0.113 * eGFR$ ;  $P = 0.003$ ). However, when the occurrence of AF was added in

the analysis, eGFR and AF only remained as predictors of LA-GS (LA-GS =  $29.3 - 7.8 * AF - 0.092 * eGFR$ ;  $P = 0.001$ ). When we restricted the above analysis to patients in sinus rhythm, similar to the entire population, only eGFR and logST2 acted as independent predictors of LA-GS (LA-GS =  $2.636 - 0.28 * \logST2 - 0.115 * eGFR$ ;  $P < 0.001$ ).

#### Association between HF symptoms and indices of LA and LV function

In order to investigate the relationship between the functional status and cardiac performance, we dichotomized our study cohort into asymptomatic patients (NYHA I,  $n = 19$ ) and those with moderate to severe symptoms (NYHA II-III,  $n = 65$ ). LA-GS and ST2 were the only markers demonstrating significant difference between the two groups (LA-GS:  $18.7 \pm 10.7$  vs.  $11.7 \pm 10.8\%$ ,  $P = 0.01$ ; ST2:  $30.2 \pm 14.1$  vs.  $42.8 \pm 29.0$  ng mL<sup>-1</sup>,  $P = 0.04$ , asymptomatic vs. symptomatic patients) (Fig. 1). On the other hand, neither the systolic (LV-GLS, SVi) nor the diastolic LV metrics ( $\beta$ , tau, EDP/EDV,  $E/e'$ ) or AV-coupling and the vascular function indices ( $E_a$ , SVRi) differ significantly between the two groups (Fig. 2). Additionally, there was no difference in NYHA class between patients in sinus and those in AF during the examination ( $P = 0.62$ ).



**Fig. 1** Left atrial strain and ST2 levels. (a) correlation between plasma soluble suppression of tumorigenicity-2 receptor (ST2) levels and left atrial strain (LA-GS) ( $r = 0.3$ ,  $P = 0.009$ ). (b) Comparison of ST2 levels between patients with LA-GS < 20% and LA-GS  $\geq$  20%.

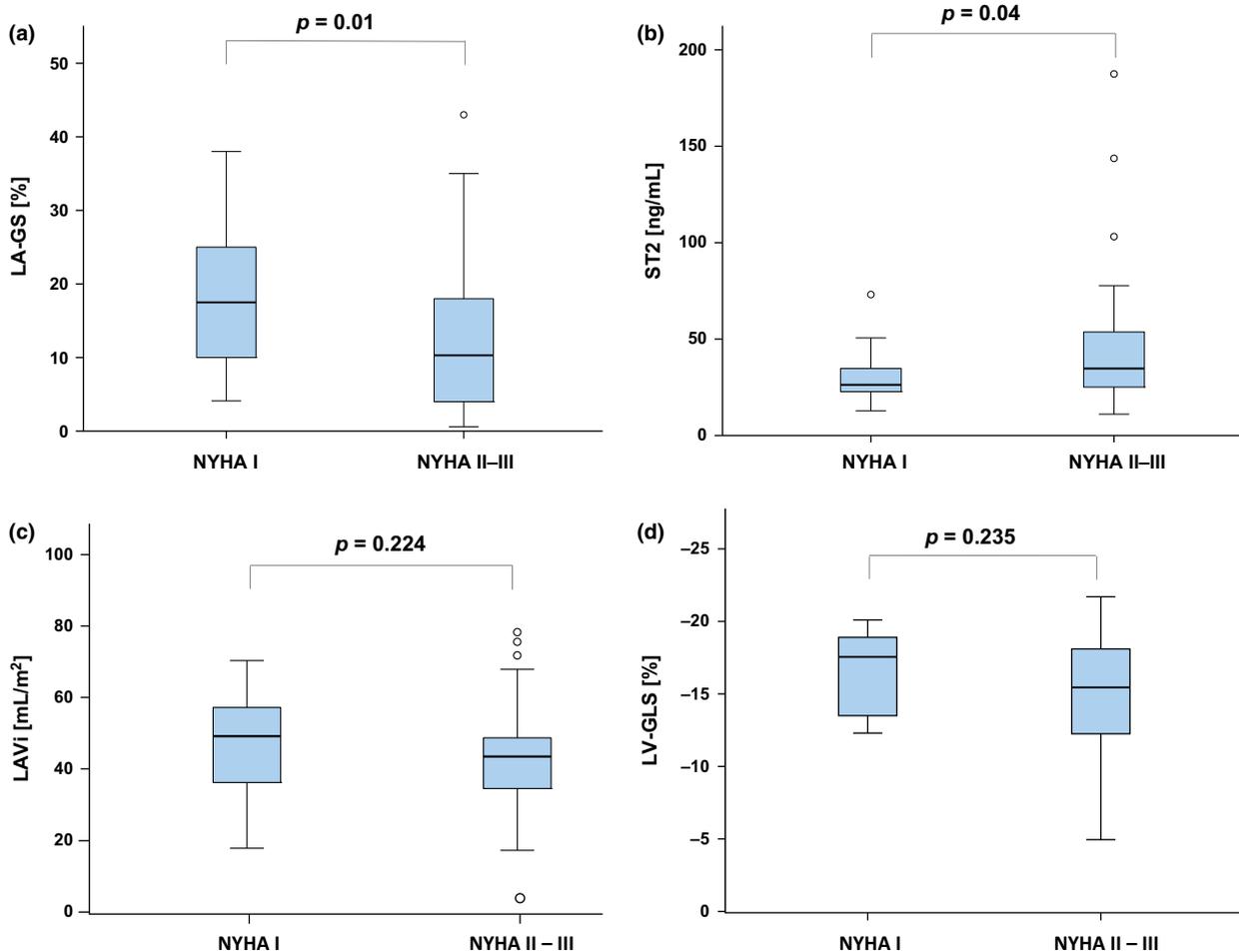
### LA strain and outcome

To investigate whether increased LA stiffness as assessed by LA-GS had a direct influence on patient prognosis, we dichotomized our patient cohort based on the LA-GS, using the third interquartile (LA-GS: 20%) as a cut-off value.

As shown in Table 2, patients with LA-GS < 20% displayed significantly lower SV<sub>i</sub>, LV-GLS and RV function along with higher  $E_a$ . Importantly,  $E/e'$  values were similar in the two groups. ST2 and NT-proBNP levels were higher in patients with more reduced LA-GS, whereas eGFR did not significantly differ between the two groups.

Over a median follow-up of 572 days (IQR: 467–1369), 32 primary outcome events occurred (five deaths, 27 first HF hospitalizations). No patients were lost to follow-up. LA-GS < 20% was associated with an increased risk of the primary composite endpoint ( $P = 0.02$ ) in unadjusted analysis [odds ratio (OR) 3.23; confidence interval (CI) 1.1–9.3,  $P = 0.029$ ]; Fig. 3a. After adjustment for age, eGFR, LV-GLS and tau, LA-GS remained an independent predictor of the outcome [OR: 4.15; CI: 1.2–14,  $P = 0.023$ ]; Fig. 3b.

As AF impacts on LA functional parameters, we proceed by further adjustment employing AF as covariate in the aforementioned regression model.



**Fig. 2** Association between heart failure symptoms, echocardiographic parameters and ST2 levels. Comparison of left atrial strain (LA-GS) (a), soluble suppression of tumorigenicity-2 receptor (ST2) levels (b), left atrial volume index (LAVi) (c) and left ventricular global longitudinal strain (LV-GLS) (d) between patient groups with (NYHA II-III) or without (NYHA I) heart failure symptoms. NYHA, New York Heart Failure Functional Classification.

LA-GS retained its significant predictive ability [OR: 4.56; CI: 1.3–15.7,  $P = 0.016$ ] although significant but weaker predictive capacity was even demonstrated for LV-GLS [ $P = 0.042$ ]; in contrast, neither the indices of AV-coupling ( $E_a$ ,  $E_{es}$ ,  $E_a/E_{es}$ ) nor the  $E/e'$  demonstrated any predictive ability for death or hospitalization. In addition, selective analysis of the patients in sinus rhythm also showed that, after adjustment for age, eGFR, tau

and LV-GLS, LA-GS remained an independent predictor of outcome in this population [OR: 4.24; CI: 1.2–14.9,  $P = 0.019$ ].

#### Discussion and conclusion

In the present prospective study, we demonstrate that in HFpEF: (i) pro-fibrotic changes as indicated by the ST2 biomarker are associated with

**Table 2** Cardiac and vascular geometric and functional measures in the two groups stratified according to LA-GS.

	Entire cohort	LA-GS < 20%	LA-GS $\geq$ 20%	P-value
BMI		29.5 $\pm$ 6.0 (57)	30.4 $\pm$ 6 (20)	NS
Age		72.8 $\pm$ 8 (59)	71.9 $\pm$ 10 (20)	NS
SBP (mmHg)		141 $\pm$ 20 (59)	148 $\pm$ 22 (20)	NS
DBP (mmHg)		78 $\pm$ 8 (59)	80 $\pm$ 11 (20)	NS
LV dimensions				
LV EDVi (mL*m <sup>-2</sup> )	56.6 $\pm$ 14 (82)	57.2 $\pm$ 15.1 (58)	55.7 $\pm$ 12 (20)	NS
LV ESVi (mL*m <sup>-2</sup> )	23.9 $\pm$ 11 (82)	25.3 $\pm$ 11.2 (58)	20 $\pm$ 7 (20)	NS
LVMi (g*m <sup>-2</sup> )	120 $\pm$ 31 (82)	119.6 $\pm$ 34 (58)	118 $\pm$ 20 (20)	NS
LV systolic function				
LVEF (%)	62.5 $\pm$ 7 (82)	61.6 $\pm$ 7.6 (58)	65.2 $\pm$ 5 (20)	NS
LV-GLS (%)	-15.3 $\pm$ 3.6 (80)	-14.9 $\pm$ 3.7 (58)	-16.9 $\pm$ 3 (18)	0.03
SVi (mL*m <sup>-2</sup> )	37.4 $\pm$ 11 (81)	35.6 $\pm$ 10.8 (58)	43.4 $\pm$ 10 (19)	0.012
$E_{es}$ (mmHg*mL <sup>-1</sup> )	2.2 $\pm$ 0.9 (81)	2.2 $\pm$ 0.9 (58)	2.1 $\pm$ 0.8 (19)	NS
LV diastolic function				
E/A ratio	1.8 $\pm$ 1.4 (60)	2.2 $\pm$ 1.6 (40)	1.1 $\pm$ 0.3 (20)	0.008
$e'$ mean	7.9 $\pm$ 2.2 (83)	8.2 $\pm$ 2.4 (58)	7.0 $\pm$ 1.5 (20)	NS
$E/e'$ mean	12.6 $\pm$ 6 (83)	12.7 $\pm$ 6.1 (57)	12.7 $\pm$ 5 (20)	NS
LVEDP (mmHg) NI	19.5 $\pm$ 3.4 (82)	19.5 $\pm$ 3.6 (57)	19.5 $\pm$ 2.7 (20)	NS
Tau (ms) NI	45 $\pm$ 15 (81)	43.4 $\pm$ 15.9 (57)	50.9 $\pm$ 10.2 (20)	NS
$\beta$	6.0 $\pm$ 0.4 (81)	5.98 $\pm$ 0.46 (57)	6.03 $\pm$ 0.4 (20)	NS
EDP/EDV (mmHg*mL <sup>-1</sup> )	0.19 $\pm$ 0.06 (81)	0.19 $\pm$ 0.1 (58)	0.18 $\pm$ 0.04 (20)	NS
Vascular function				
$E_a$	1.96 $\pm$ 0.8 (81)	2.1 $\pm$ 0.9 (58)	1.6 $\pm$ 0.3 (19)	0.035
$E_a/E_{es}$	1.0 $\pm$ 0.42 (81)	1.1 $\pm$ 0.4 (58)	0.8 $\pm$ 0.3 (19)	NS
SVRi (mmHg*L <sup>-1</sup> *m <sup>-2</sup> )	47.4 $\pm$ 15 (83)	48 $\pm$ 16 (58)	46.7 $\pm$ 13.2 (20)	NS
Arterial compliance (mL*mmHg <sup>-1</sup> )	0.72 $\pm$ 0.22 (81)	0.69 $\pm$ 0.2 (58)	0.83 $\pm$ 0.2 (19)	NS
LA function				
LA ESVi (mL*m <sup>-2</sup> )	44.4 $\pm$ 16 (79)	44.4 $\pm$ 17.1 (59)	44.2 $\pm$ 14 (20)	NS
LA-GS (%)	13.3 $\pm$ 11 (79)	8.1 $\pm$ 6.3 (59)	28.7 $\pm$ 7 (20)	<0.0001
LA EF (%)	28.6 $\pm$ 18.4 (79)	21.7 $\pm$ 15.2 (59)	49.1 $\pm$ 10.1 (20)	<0.001
RV function				
TAPSE (mm)	16.5 $\pm$ 4 (83)	15.7 $\pm$ 4.1 (58)	19.1 $\pm$ 4 (20)	0.003

Table 2 (Continued)

	Entire cohort	LA-GS < 20%	LA-GS ≥ 20%	P-value
Biochemical				
NT-proBNP (ng*L <sup>-1</sup> )		1400 (Q1:556; Q3:2633)	495 (Q1:430; Q3:822)	0.003
ST2 (ng*mL <sup>-1</sup> )		35.0 (Q1:25; Q3:55)	26.7 (Q1:19; Q3:33)	0.003
eGFR (mL*min <sup>-1</sup> /1.73 m <sup>2</sup> )		70 (Q1:56; Q3:86)	59.5 (Q1:45; Q3:80)	NS

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LV, left ventricle; EDVi, end-diastolic volume index; ESVi, end-systolic volume index; LVMI, LV mass index; EF, ejection fraction; LV-GLS, LV global longitudinal strain; SVi, stroke volume index;  $E_{es}$ , LV end-systolic elastance;  $E/A$ , ratio between the early diastolic inflow velocity ( $E$ ) to the inflow velocity due to atrial contraction ( $A$ );  $e'$  mean, mean value of early myocardial velocity in LV basal septal and lateral wall;  $E/e'$ , ratio between the  $E$  and the  $e'$ ; EDP, end-diastolic pressure; NI, noninvasive; tau, time constant of LV isovolumic relaxation;  $\beta$ , diastolic stiffness constant describing the steepness of the EDPVR curve; EDP/EDV, end-diastolic pressure-to-end-diastolic volume ratio;  $E_a$ , effective arterial elastance; SVRI, systemic vascular resistance index; LA, left atrium; LA ESVi, left atrial end-systolic volume; LA-GS, left atrial global longitudinal strain; LA EF, left atrial ejection fraction; TAPSE, tricuspid annular plane systolic excursion; NT-proBNP, N-terminal pro-brain natriuretic peptide; ST2, soluble suppression of tumorigenicity-2 receptor; eGFR, estimated glomerular filtration rate; NS, nonsignificant ( $P \geq 0.05$ ). Data are provided as mean  $\pm$  SD followed by patient number in brackets; or median values followed by 1st and 3rd quartiles in brackets.

mechanical alterations of the LA but not the LV; and (ii) the LA strain comprised an independent predictor of death or hospitalization independently of the degree of LV remodelling or dysfunction.

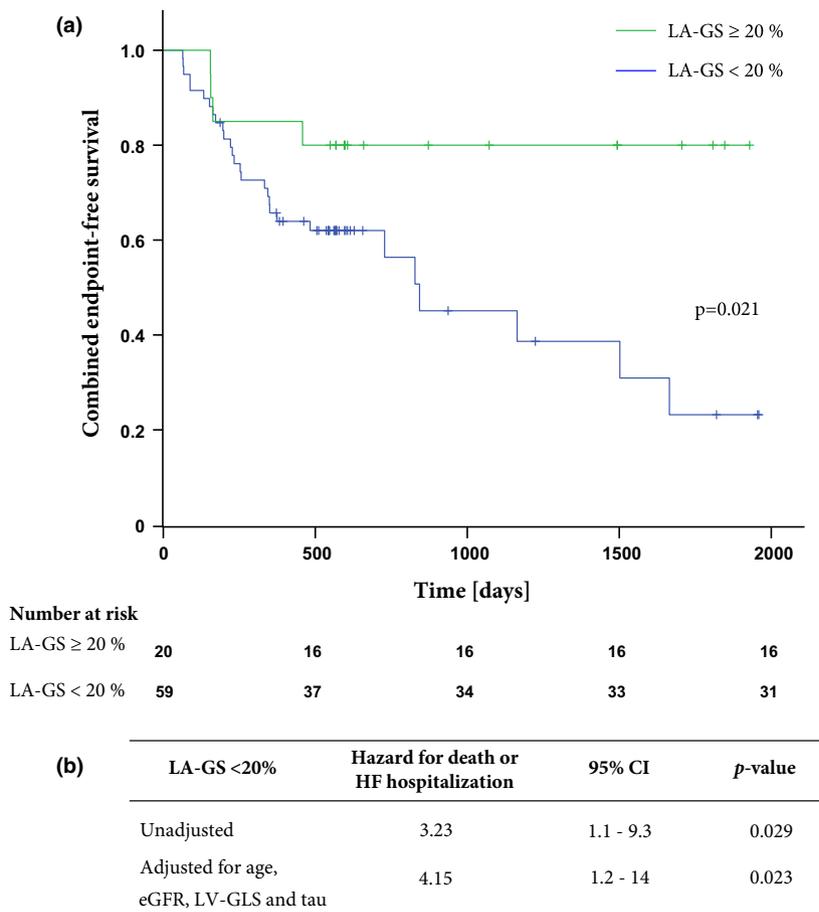
The LA reservoir function is influenced by both LV systolic function and the intrinsic LA compliance and plays an important role in disease progression in various pathologies including AF, acute myocardial ischaemia and HF. LA strain is an emerging noninvasive method for the quantification of LA reservoir function [6].

Left atrium strain has been shown to reflect the extent of LA fibrosis in various pathological states. Kuppahally *et al.* [22] assessed the degree of LA wall fibrosis by delayed-enhancement MRI in AF patients and found that LA strain inversely associated with the degree of LA fibrosis. In another report, in patients undergoing mitral valve surgery, preoperatively measured LA strain was the strongest independent predictor of the degree of histopathologically quantified LA wall fibrosis [23].

In our study, ST2, an established pro-fibrotic marker, significantly associated with LA-GS. Conversely, measures of the systolic, early diastolic and late diastolic LV function or those of the AV-coupling and vascular function were not related to ST2 levels. In agreement with our results, previous studies in HFpEF failed to demonstrate any relationship between ST2 levels and LV echocardiographic parameters [14, 24]. Even though ST2 has

repeatedly been shown to be a reliable marker of disease severity in HFpEF, the aforementioned observation has led to the misconception that elevated ST2 might barely indicate systemic inflammation, rather than reflect direct cardiac alterations [14, 25]. No studies, however, have specifically investigated the association between LA function and ST2 levels in HFpEF.

The LV contraction towards the apex is expected to act as a major determinant of the LA deformation during systole [26]. In our cohort, however, no association between the LV longitudinal deformation and LA-GS was found. This might be explained by disparate responses of the LA and the LV to inflammation, as myocardial remodelling at the atrial level has been shown to involve differential pathophysiologic pathways from the LV. In a tachycardia-induced HF model, considerably different cellular responses were observed in these two chambers, with more pronounced inflammatory and pro-fibrotic reaction detected in the LA as compared to the LV wall [27]. In another study, angiotensin II infusion resulted in progressive LA fibrosis that was independent of LV wall stress but directly related to circulating hormone levels [28]. Conceivably, due to its thinner wall, the LA might be more susceptible to myocardial fibrosis and exhibit more apparent mechanical changes as compared to LV. Our results advocate that LA-GS comprises a surrogate marker of LA mechanical changes partly ascribed to a pro-fibrotic reaction and imply that LA structural and functional



**Fig. 3** Left atrial strain and patient outcome. (a) Kaplan–Meier analysis of patients stratified by left atrial strain (LA-GS). Group I, LA-GS < 20%; Group II, LA-GS  $\geq$  20%. (b) Hazard ratio for death or heart failure (HF) hospitalization for patients with LA-GS < 20% compared to LA-GS  $\geq$  20%. eGFR, estimated glomerular filtration rate; LV-GLS, left ventricular global longitudinal strain; tau, time constant of LV isovolumic relaxation; CI, confidence interval.

abnormalities might develop on the ground of intrinsic LA alterations, independently of LV dysfunction.

Normally, the distensible LA accommodates blood from the pulmonary veins without a considerable rise in the LA pressure (LAP). In cases of reduced LA compliance, as in LA fibrosis, the LA pressure–volume curve is shifted upwards resulting in disproportional rise of LAP for the same volume entering the chamber. Chronically elevated LAP leads to LA enlargement. Based on physical principles, increased LA volume would mitigate the elevated wall stress and accordingly the LAP. Paradoxically however, the degree of LA remodelling has been shown to be positively related to the

severity of pulmonary hypertension [29]. This observation can be physiologically explained by concomitantly occurring LA wall fibrosis, which counteracts the alleviating effect of LA volume increase. Of note, in this study, LAVi was not associated with either ST2 levels or the LA-GS. Similar findings were also reported by others, suggesting that the degree of LA fibrosis is not solely or even primarily determined by the degree of LA enlargement [6, 10], which supports our hypothesis that LA fibrosis, as reflected by elevated ST2, might be a result of an inflammatory process rather than haemodynamic overload. In our study, LA enlargement was not associated with the patients' functional class either, whereas a significant relationship between LA-GS and symptom

severity was observed. Similar association was also found between ST2 levels and symptomatology, further supporting the notion of the potential impact of inflammatory activity on LA mechanics.

LV diastolic properties have been extensively studied in regard to their association with symptoms in HFpEF. Employing measurements with conductance catheters, Liu *et al.* [4] demonstrated that HFpEF patients display increased LV end-diastolic stiffness and impaired relaxation as compared to healthy controls. These findings were confirmed in a larger scale study in which noninvasive estimates of LV relaxation and stiffness were used [8]. In another report, however, early relaxation and LV end-diastolic stiffness were similar between HFpEF patients and healthy subjects at rest [30] and elevated EDP in HFpEF was not accompanied by increased  $\beta$  values advocating for the influence of extracardiac forces rather than passive LV stiffness on the elevated filling pressures. In our study, indices of LV relaxation and diastolic stiffness did not correlate with ST2 levels. However, there was a weak association between tau and LA-GS suggesting that apart from fibrotic changes, mechanical alterations in the LV function during the relaxation phase may also influence the LA mechanics.

#### *LA strain and prognosis*

To date, few studies have attempted to assess the predictive value of LA function regarding outcome in HFpEF. Recently, Melenovsky *et al.* [7] demonstrated that LA EF was an independent predictor of mortality in HFpEF. In another study, Santos and colleagues showed that reduced LA strain implied an increased risk of HF hospitalization in HFpEF patients; however, it did not remain prognostic after adjustment for LV deformation and the  $E/e'$  [31]. Accordingly, the authors conclude that the predictive ability of LA strain is to be attributed merely to its association with LV performance [31]. Importantly, our findings contrast this observation as we show that LA-GS independently predicted outcome even when adjusted for measurements of LV longitudinal systolic deformation and diastolic performance as well as for indices of atrioventricular coupling. These disparities might be explained by differences in patient profile. Our cohort consisted of older patients, in whom the structural and functional LA indices as well as the higher prevalence of AF indicated more advanced disease. Also, in the present work, a dedicated software for LA strain analysis was employed, presumably yielding

more representative measurements, as compared to earlier studies that applied LV strain measuring algorithm for LA strain analysis.

Importantly, in our study other well-established indices such as those describing the AV-coupling and the  $E/e'$  did not demonstrate significant prognostic value. The later noninvasive marker of LA filling pressures has been shown to entail significant predictive value in HFpEF. Our findings indicate that LA strain, reflecting not merely the haemodynamic filling state but, as previously discussed, also the degree of pro-fibrotic alterations, constitutes a more robust marker of disease severity in this clinical condition.

AF is a common condition in HFpEF that importantly influences LA-GS measurements and thus could be a concern for the reliability and utility of LA-GS measurement in these patients. In order to rule out the confounding effect of AF on LA-GS measurement, we tested the association of ST2 with LA-GS confining our analysis to patients in sinus rhythm. ST2 showed a significant correlation with LA-GS in these patients also. Similarly, the independent prognostic value of LA-GS for outcome was maintained in this subgroup of patients.

#### *Limitations*

LA strain is a more and more widely used noninvasive metric of LA reservoir function; however, the actual haemodynamic meaning of LA strain is rather ambiguous. Although the LA deformation during systole is expected to represent a surrogate of LA compliance, LA strain is also influenced by other components of the cardiac mechanics. A more accurate assessment of the LA reservoir function would require direct measurement of LA stiffness by an invasive approach, preferably with micromanometric catheters. On the other hand, the obvious correlations found with both ST2 levels and patient outcome speak for the utility of LA strain, as a readily obtainable metric providing clinically important information. The relatively limited size of our study, as well as the fact, that of the 32 outcome events that occurred during follow-up, 27 were heart failure hospitalizations, warrants larger scale investigations to confirm our results. At the same time, the prognostic information evident even at this patient number corroborates the clinical significance of our findings.

## Conclusions

Our results indicate that ST2 is a sensitive marker of LA dysfunction in HFpEF, elevation of which may specifically reflect LA mechanical alterations, independently of LV performance. Impaired LA strain remained an independent predictor of HF hospitalization and mortality, even after adjustment for clinical variables and LV functional indices.

Considering the fact that currently no effective therapy for HFpEF is available, a reliable tool for monitoring the evolution of this disease is of major clinical significance for the timely recognition and thus prevention of patients at increased risk, prior to the development of irreversible LA remodelling.

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## Conflict of Interest

None.

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Correspondence: Anikó Ilona Nagy, Semmelweis University, Heart and Vascular Center, 68. Városmajor u., 1122 Budapest, Hungary.  
(phone: +36-20-8259738; e-mail: anychophora@gmail.com)

### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Diagram illustrating the temporal sequence of study events. ■