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Association of fibrotic markers with diastolic function after STEMI

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Galectin-3 and Suppression of tumorigenicity-2 (ST2) are known markers of cardiac fibrosis. We investigated the prognostic value of fibrotic markers for the development of diastolic dysfunction and long-term outcome in patients suffering an ST-elevated myocardial infarction (STEMI). We analyzed 236 patients from the GIPS-III cohort with available echocardiographic studies and plasma measurements at hospitalization and after 4 months follow-up. Adjusted logistic mixed effects modelling revealed no association between the occurrence of diastolic dysfunction over time with abnormal plasma levels of galectin-3 and ST2. We observed no differences regarding survival outcome at follow-up of 5 years between patients with normal versus abnormal values in both galectin-3 ($P = 0.75$), and ST2 ($P = 0.85$). In conclusion, galectin-3 and sST2 were not associated with the development of diastolic dysfunction in non-diabetic patients that presented with a STEMI.

Abbreviations

ST2	Suppression of tumorigenicity
sST2	Soluble suppression of tumorigenicity
ST2L	Transmembrane suppression of tumorigenicity
STEMI	ST-elevated myocardial infarction
MACE	Major adverse cardiac events
IL	Interleukin
ST2L	Transmembrane suppression of tumorigenicity
CRD	C-terminal carbohydrate-recognition domain
LVEF	Left ventricular ejection fraction
PCI	Percutaneous coronary intervention
BSA	Body surface area
LAV	Left atrial volume
GLS	Left ventricular global longitudinal strain
E	Mitral valve early filling flow
e′	Early diastolic tissue velocity
E/e′	Ratio of transmitral early flow to early mitral annulus velocity
SD	Standard deviation
IQR	Interquartile range
LVMI	Left ventricular mass index
TIMI	Thrombolysis in myocardial infarction
HbA1c	Glycated hemoglobin
NT-proBNP	N-terminal pro brain natriuretic peptide
LDH	Lactate dehydrogenase
eGFR	Estimated glomerular filtration rate
CK	Creatine kinase
CK–MB	Creatine kinase–Myocardial band
ASAT	Aspartate aminotransferase
LDL	Low density lipoprotein
HDL	High density lipoprotein

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Over the past decades, innovations in standard care for patients suffering ST-elevated myocardial infarction (STEMI) have led to improved survival rates. Simultaneously, the incidence of comorbidities such as heart failure have increased in the aftermath of STEMI. A hallmark of heart failure is fibrosis which occurs both as reparative and reactive response to myocardial infarction[1].

Galectin-3 and Suppression of tumorigenicity 2 (ST2) are known markers of cardiac fibrosis. ST2 is a member of the interleukin (IL)-1 receptor family. The primary cell source of ST2 has not yet been discovered, however there are several indications that vascular endothelial cells might be an important source of ST2. It is also suggested as a possible therapeutic target[2]. Soluble suppression of tumorigenicity (sST2) and the isoform transmembrane suppression of tumorigenicity (ST2L) are mostly studied in heart failure research[3]. ST2 is upregulated by cardiomyocytes and cardiac fibroblasts when mechanical stress is imposed, for instance stretch.

Galectin-3 is a beta-galactoside binding lectin that is produced by macrophages during myocardial stress and activates fibroblasts[4]. Galectin-3 consists of two domains, namely an atypical N-terminal domain and a C-terminal carbohydrate-recognition domain (CRD). During differentiation of monocytes into macrophages galectin-3 is released and engages in many processes during the acute inflammatory response such as neutrophil activation and chemoattraction of monocytes. Galectin-3 has been identified as a causal factor in the development of cardiac fibrosis[5]. Furthermore, galectin-3 is associated with outcome (mortality/heart failure rehospitalization) in different sub-set of patients (ischemic, non-ischemic) and in the general population[6,7].

Both galectin-3 and sST2 are thought to be promising markers for risk stratification in heart failure patients. However, our knowledge of their prognostic value in a post-STEMI setting is limited. We previously reported that galectin-3, but not sST2, taken immediately after STEMI, predicts left ventricular ejection fraction (LVEF) and infarct size after 4 months[8]. The association of (change in) these fibrotic markers with (change in) diastolic function and long-term outcome after STEMI remains unknown. In this study we therefore aim to investigate the prognostic value of fibrotic markers for predicting diastolic dysfunction and long-term outcome in non-diabetic patients suffering a first STEMI.

Methods

This is a sub-study of the GIPS-III trial, a single center, randomized, double-blind, placebo-controlled study about the effect of metformin on LVEF. GIPS-III is registered as a clinical trial with identifier: NCT01217307. Design of this study, patient characteristics at hospitalization and primary outcomes have been reported previously[9–11]. Briefly, non-diabetic patients aged 18 years and over presenting to the University Medical Center Groningen with STEMI were randomized to metformin or placebo treatment after successful percutaneous coronary intervention (PCI) with implantation of at least 1 stent with a diameter of at least 3 mm were included. Important exclusion criteria were inability to undergo magnetic resonance imaging, previous myocardial infarction, and severe renal dysfunction. Metformin 500 mg twice daily or visually matching placebo twice daily was started immediately after PCI and lasted 4 months. All patients were contacted by phone at 5 years after STEMI to determine long term outcome. Patients provided verbal informed consent before PCI and written consent following admission to the Coronary Care Unit. The study protocol was approved by the local ethics committee of the University Medical Center Groningen (Groningen, the Netherlands), and was in accordance with the Declaration of Helsinki and Dutch laws.

Measurements of biomarkers

On hospital admission and 4 months after acute STEMI blood samples were obtained and anticoagulated with EDTA. These samples were spun down and plasma was stored at – 80 °C until further assaying. Galectin-3 levels were measured in the plasma samples with the FDA-cleared galectin-3 ELISA kit (BG Medicine, Waltham, MA). Intra- and inter-assay coefficient of variability of this assay is 3.2% and 5.6% respectively[12]. This assay has been described in greater detail previously[13]. sST2 was measured in the plasma samples with the FDA-cleared Presage® ST2 Assay (Critical Diagnostics, San Diego, CA). Intra- and inter-assay coefficient of variability of this assay is 5.1% and 5.2% respectively. Further characteristics of this assay have been published elsewhere[14]. FDA-cleared threshold values of 17.8 ng/mL for galectin-3 and 35.0 ng/mL for sST2 were used to categorize patients in groups of elevated versus non-elevated plasma levels. Delta biomarker levels were calculated as the 4-month level minus the hospitalization level and used to categorize patients in groups with decreased versus increased plasma levels. All other samples were measured in a routine setting. Creatinine (enzymatic), CK and CK-MB activity was measured on a Roche Modular P platform (Roche, Mannheim, Germany). NT-proBNP and Troponin T were measured on a Roche Modular E system (Roche, Mannheim, Germany). Troponin T was determined using either a fourth or a fifth-generation troponin assay.

Echocardiographic assessment

During hospitalization for the index event and at 4 months follow-up, trans-thoracic echocardiogram was performed in left decubital position using a Vivid 7 echo system (General Electric, Horton, Norway). All echocardiographic data were digitally stored in DICOM format and off-line analyses were performed on an Echopac BT 10 (General Electric, Horton, Norway) at an independent core lab (Groningen Imaging Core Laboratory, Groningen, the Netherlands). These analyses were performed by observers that were blinded to treatment allocation and all other patient data. Evaluation of the echocardiographic data was performed in accordance to the contemporary guidelines[15–17]. When appropriate, echocardiographic measures were indexed for body surface area (BSA) according to the formula of Du Bois and Du Bois[18].

In concordance with the latest recommendations from the Heart Failure Association of the European Society of Cardiology for diagnosing heart failure with preserved ejection fraction[19], the following measurements related to diastolic function were assessed: Left atrial volume (LAV) was measured with the area length method.

The left ventricular mass was estimated from linear dimensions as suggested by Devereux and colleagues[17]. Left ventricular global longitudinal strain (GLS) assessments for the left ventricle were performed in the apical four-, three-, and two-chamber views using speckle-tracking. Doppler measurements were used for the mitral valve early filling flow (E). Using tissue color Doppler, early diastolic tissue velocities (e′) from both the septal and lateral wall were measured[16,20]. Mean e′ was calculated as (e′ septal + e′ lateral)/2. The ratio of trans mitral early flow to early mitral annulus velocity (E/e′) was calculated as E/mean e′. Reported values represent the mean of three heart beats in end-expiration.

Study outcomes

Diastolic dysfunction was defined as a multi-parametric dichotomous value based on the recommendations from the Heart Failure Association of the European Society of Cardiology for diagnosing heart failure with preserved ejection fraction (HFA-PEFF score)[19]. The HFA-PEFF score has functional, morphological, and biomarker domains. Within each domain, a major criterion is worth 2 points, and a minor criterion is worth 1 point (Supplementary Fig. 1). Major and minor criteria are not additive in a single domain and points are added only when they come from different domains. In our study a total score ≥ 5 points was considered to be diagnostic for diastolic dysfunction. A separate categorical value was composed to identify patients in whom diastolic function either improved, deteriorated, or remained unchanged between hospitalization and follow-up.

Additionally, the presence of abnormal individual values for echocardiographic measurements used to determine diastolic function were compared between groups with non-elevated versus elevated fibrotic markers. Abnormal values were defined based on clinically established cut off points[19].

The fibrotic marker groups were formed based on the FDA appointed thresholds plasma levels for galectin-3 (> 17.8 ng/mL) and sST2 (> 35.0 ng/mL).

Major adverse cardiac events (MACE), defined as death, re-infarction, and target lesion revascularization, was the primary parameter for long term outcome.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or median (interquartile range, IQR) for normally and non-normally distributed data, respectively. Categorical variables are presented as frequency (percentage). Non-normally distributed data was transformed using log-transformation. Outcome observations of median ± 5 times the standard deviation was considered extreme outliers and excluded from the cohort. Differences between groups were tested using two-tailed t test for normally distributed data and Wilcoxon rank-sum for non-normally distributed data. Reported *P* values are two-sided. Predictors of plasma levels for galectin-3 and sST2 at hospitalization were determined using regression models to determine relevant co-variates for subsequent analyses. If correlation between these predicting variables was present, the strongest predictor (greatest β-coefficient) was selected for further use.

We utilized logistic mixed model analysis to analyze the association of diastolic dysfunction over time with elevated plasma levels of galectin-3 and sST2 at hospitalization, 4 months follow-up, and categorical change between hospitalization and 4 months follow-up (improved, deteriorated, or unchanged), with random effects factor for each individual patient subject. These models were adjusted for variables that were significant predictors of galectin-3 and sST2 at hospitalization (leukocytes, estimated glomerular filtration rate, urea, total cholesterol, myocardial band of creatine kinase (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT), N-terminal pro–B-type natriuretic peptide (NT-proBNP), ischemic time, and TIMI flow grade post PCI < 3). Models were additionally adjusted for the GIPS-III treatment intervention variable (metformin use), and variables that are known to be associated with diastolic dysfunction (age, sex, and history of hypertension).

To reduce the chance of a type I error in this explorative biomarker study a *P* value of < 0.005 was considered to indicate a significant association or difference between groups, and a *P* value between 0.05 and 0.005 was considered suggestive in accordance with the position paper by Benjamin et al.[21]. When *P* > 0.10 only 2 decimal points are shown for clarity.

A multivariable Cox proportional hazard model for was constructed to test equality of survivor functions across groups with non-elevated versus elevated fibrotic markers. Analyses were performed using Stata version 18.0 (StataCorp), and R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical approval

The study protocol was approved by the local ethics committee (Groningen, the Netherlands), and was in accordance with the Declaration of Helsinki and Dutch laws. Informed consent was obtained from all patients that were included in this study.

Results

Study population

All 379 patients participating in the GIPS-III trial were alive at 4 months. Echocardiographic assessment and plasma levels of fibrotic markers could be determined in 306 (81%) patients during hospitalization and 290 (77%) patients at 4 months. 238 (63%) patients with echocardiographic assessment and fibrotic markers at both time points were eligible for our study analyses. Of these, 2 (1%) patients were recognized as having extreme outliers and excluded from the cohort. 236 (62%) patients with reliable echocardiographic assessments and fibrotic markers remained eligible for the final analysis (Supplementary Fig. 2).

Mean age of the 236 included patients was 57.9 ± 11.4 years and 21.6% were females. The characteristics at hospitalization were compared between the patients with diastolic dysfunction and those without diastolic dysfunction (Table 1). Patients with diastolic dysfunction were significantly older (*P* < 0.001), were more likely

		No. (%)		
Characteristic	**Total (n = 236)**	**No Diastolic dysfunction (n = 163)**	**Diastolic dysfunction (n = 73)**	***P*-value**
Age, years	57.9 ± 11.4	55.9 ± 10.6	62.3 ± 12.0	**< 0.001**
Women	51 (21.6%)	32 (19.6%)	19 (26.0%)	0.27
Metformin treatment	123 (52.1%)	85 (52.1%)	38 (52.1%)	0.99
Body-mass Index, kg/m^2	26.9 ± 3.6	27.0 ± 3.8	26.5 ± 3.1	0.30
Race/ethnicity				
White	227 (96.2%)	157 (96.3%)	70 (95.9%)	0.87
Asian	8 (3.4%)	5 (3.1%)	3 (4.1%)	0.68
Black	1 (0.4%)	1 (0.6%)	0 (0.0%)	1.00
Cardiovascular related history				
Hypertension	63 (26.7%)	33 (20.2%)	30 (41.1%)	**< 0.001**
Dyslipidemia	142 (60.2%)	105 (64.4%)	37 (50.7%)	0.046
Current smoking	129 (54.7%)	91 (55.8%)	38 (52.1%)	0.59
Stroke	2 (0.8%)	2 (1.2%)	0 (0.0%)	1.00
Previous PCI	2 (0.8%)	1 (0.6%)	1 (1.4%)	0.56
Blood pressure, mmHg				
Systolic	134.4 ± 23.1	132.6 ± 20.3	138.3 ± 28.3	0.077
Diastolic	84.8 ± 14.7	83.8 ± 14.1	87.0 ± 16.0	0.13
Heart rate, beats/min	75.6 ± 15.5	75.1 ± 16.2	76.6 ± 13.8	0.49
Infarct-related factors				
Ischemia time, min	153 (108, 245)	148 (105, 236)	161 (110, 264)	0.28
Multi vessel disease	170 (72.0%)	119 (73.0%)	51 (69.9%)	0.62
Anterior infarction	88 (37.3%)	53 (32.5%)	35 (47.9%)	0.023
Intervention-related assessments				
TIMI flow grade pre-PCI ≤ 1	155 (65.7%)	99 (60.7%)	56 (76.7%)	0.017
TIMI flow grade post-PCI < 3	18 (7.6%)	11 (6.7%)	7 (9.6%)	0.45
Myocardial blush grade ≤ 1	20 (8.6%)	12 (7.5%)	8 (11.1%)	0.36
Laboratory values at admission				
Glucose, mmol/L	8.1 (7, 9.5)	8.1 (6.8, 9.2)	8.6 (7.3, 10.1)	0.11
HbA_{1c}, %	5.8 (5.6, 6)	5.75 (5.6, 6)	5.8 (5.6, 6.1)	0.15
Hemoglobin, mmol/L	8.9 (8.45, 9.4)	9 (8.4, 9.4)	8.9 (8.5, 9.3)	0.57
Creatinine, µmol/L	72 (64, 82)	74 (64, 84)	70 (60, 79)	0.024
eGFR, ml/min/1.73m^2	95 (86, 103)	96 (86, 103)	93 (86, 102)	0.38
NT-proBNP, ng/L	72 (36, 171)	54 (32, 115)	112 (66, 315)	**< 0.001**
CK, U/L	124 (84, 207)	118 (81, 189)	149 (93, 293)	0.074
Myocardial band of CK, U/L	15 (13, 23)	15 (12, 21)	16 (13, 32)	0.051
Troponin T, ng/L	45 (20, 136)	40 (17, 124)	57 (29, 204)	0.017
Total cholesterol, mmol/ L	5.4 (4.8, 6.1)	5.5 (4.9, 6.2)	5.2 (4.55, 5.9)	0.035
LDL cholesterol, mmol/ L	3.8 (3.2, 4.5)	3.9 (3.3, 4.6)	3.65 (3.1, 4.2)	0.049
HDL cholesterol, mmol/L	1.1 (0.9, 1.3)	1.1 (0.9, 1.3)	1.2 (1.0, 1.4)	0.028
Galectin-3, ng/mL	13.2 (11.2, 15.8)	12.9 (11.1, 15.2)	14.2 (11.2, 16.2)	0.14
sST2, ng/mL	30.1 (24.8, 39.6)	29.7 (24.6, 39.2)	30.9 (26.1, 43.9)	0.25

Table 1. Baseline characteristics of all patients, and stratified by presence of diastolic dysfunction at 4 months after STEMI. PCI, percutaneous coronary intervention; IQR, interquartile range; TIMI, Thrombolysis in Myocardial Infarction; HbA1c, glycated hemoglobin; NT-proBNP, N-terminal pro brain natriuretic peptide; eGFR, estimated glomerular filtration rate; CK, creatine kinase; LDL, low density lipoprotein; HDL high density lipoprotein; sST2, Soluble suppression of tumorigenicity.

to have a history of hypertension ($P = 0.002$) and had significantly higher plasma levels of NT-proBNP at index event ($P < 0.001$) as compared to those without diastolic dysfunction.

Diastolic dysfunction and fibrotic markers over time

At hospitalization, 39 (17%) patients had diastolic dysfunction, which increased significantly over time to 73 (31%) patients at 4 months ($P < 0.001$; Fig. 1).

Plasma levels of the fibrotic biomarkers of patients with a first STEMI were relatively stable over time (Fig. 3). Median levels of galectin-3 at hospitalization were 13.2 (11.2–15.8) ng/mL and were comparable after 4 months

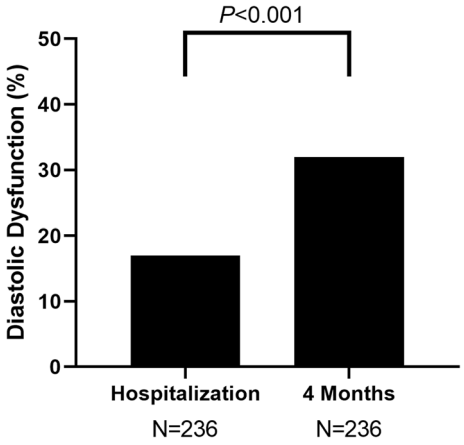

Figure 1. Prevalence of diastolic dysfunction over time.

(13.6 ng/mL (11.6–15.9); $P = 0.050$; Fig. 2A). The median change between hospitalization and 4 months follow up was 0.6 (− 1.3 to 2.7) ng/mL. Galectin-3 levels were elevated (≥ 17.8 ng/mL) in 33 (14.0%) patients at hospitalization and in 35 (14.8%) patients at follow-up. Elevated galectin-3 levels improved to normal in 15 (6.4%) patients, whereas normal levels of galectin-3 deteriorated to elevated levels in 17 (7.2%) patients.

Median levels of sST2 at hospitalization were 30.1 (24.8–39.6) ng/mL and decreased numerically ($P < 0.001$) to 29.1 ng/mL (22.9–35.1) after 4 months (Fig. 2B). The median change between hospitalization and 4 months follow up was − 1.4 (− 7.3 to 2.9) ng/mL. sST2 levels were elevated (≥ 17.8 ng/mL) in 85 (36.0%) patients at hospitalization and in 60 (25.4%) patients at follow-up. Elevated sST2 levels improved to normal in 38 (16.1%) patients, whereas normal levels of sST2 deteriorated to elevated levels in 13 (5.5%) patients.

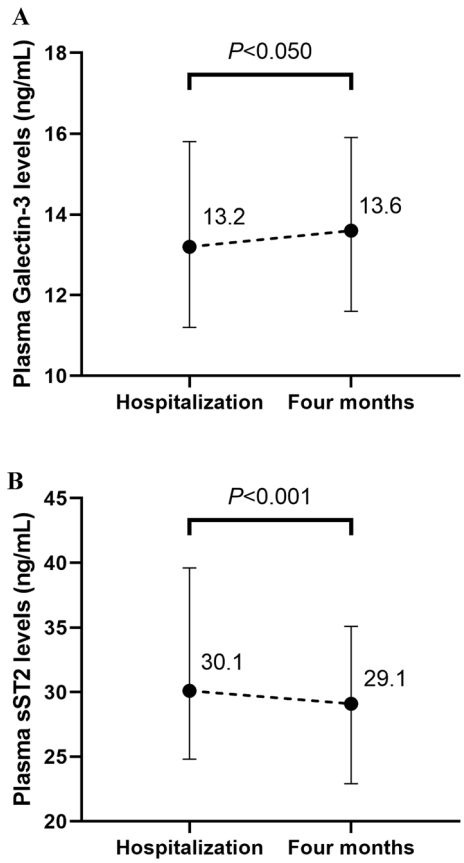

Figure 2. Biomarker levels over time. Median levels of serum galectin-3 (**A**) and serum sST2 (**B**) after acute STEMI, at hospitalization and after 4 months, are shown. Whiskers represent interquartile ranges.

Elevated levels of galectin-3 and sST2 were not associated with individual echocardiographic parameters of diastolic dysfunction at either hospitalization or 4 months follow-up (Table 2).

Association between galectin-3 and sST2, and development of diastolic dysfunction

Adjusted logistic mixed effects modelling revealed no association between the occurrence of diastolic dysfunction over time with abnormal plasma levels of galectin-3 (Fig. 3) and sST2 (Fig. 4) at hospitalization, at 4 months, or with categorical change between hospitalization and 4 months follow-up.

Association of fibrotic markers with long term outcome

At 5-years follow-up, 20 (8%) patients had experienced a MACE event. A multivariable Cox proportional hazard model adjusting for variables associated with diastolic dysfunction, galectine-3, and sST2 revealed no differences regarding outcome between high and low groups in both galectin-3 ($P = 0.75$), and sST2 ($P = 0.85$; Supplementary Table 1).

Discussion

The present study suggests that galectin-3 and sST2 do not predict the development of diastolic dysfunction 4 months after STEMI. Additionally, both fibrotic markers did not predict MACE after 5 years in this patient cohort.

Diastolic dysfunction is increasingly recognized as a cause of symptomatic heart failure[22]. Diastolic dysfunction is associated with chronic hypertension, ischemic heart disease and degenerative processes. An underlying pathophysiology in each of these diseases is a dis-balance of adverse extra-cellular matrix accumulation and structural remodeling in the myocardium, referred to as cardiac fibrosis[23].

In STEMI patients it could be speculated that a time-dependent damage to both myocytes and extracellular matrix (ECM) occurs in the infarct zone post myocardial infarction. Scar tissue will form at the place of injury that is characterized by fibrotic repair. The non-infarct zone exhibits reactive hypertrophy, interstitial fibrosis and increased collagen, potentially leading to cardiac dysfunction[24–26]. Fibrosis, the hallmark of this remodeling process could be a potential target for therapy. Identification of patients at risk for adverse remodeling and fibrosis which could lead to diastolic dysfunction would help clinicians to tailor therapy with anti-fibrotic agents[27,28]. Biomarkers that reflect myocardial damage, like cardiac troponin and creatine kinase can be used to estimate infarct size early after STEMI, but their sensitivity to detect other processes in cardiac remodeling is limited.

Galectin-3 and sST2 are emerging fibrotic biomarkers that are thought to be causally involved in the development of heart failure. However, galectin-3 is not cardiac specific, but is expressed in several fibrotic and inflammatory diseases[29].

Variable	During hospitalization			At 4 months		
	Non-elevated levels	Elevated levels		Non-elevated levels	Elevated levels	
Galectin-3	**(n = 203)**	**(n = 33)**	***P*-value**	**(n = 201)**	**(n = 35)**	***P*-value**
Time until echo, days	2 (1, 3)	2 (1, 3)	0.95	123 (118, 128)	123 (117, 127)	0.67
Metformin treatment	106 (52.2%)	17 (51.5%)	0.94	101 (50.2%)	22 (62.9%)	0.17
Septal e′ < 7 cm/s	74 (36.5%)	15 (45.5%)	0.32	59 (29.4%)	14 (40.0%)	0.21
Lateral e′ < 10 cm/s	107 (52.7%)	22 (66.7%)	0.14	89 (44.3%)	19 (54.3%)	0.27
E/e′ ratio > 14	3 (1.5%)	1 (3.0%)	0.52	4 (2.0%)	2 (5.7%)	0.20
GLS < 16%	73 (65.2%)	14 (77.8%)	0.29	69 (46.6%)	10 (47.6%)	0.93
LAVI > 34 ml/m^2	29 (16.7%)	6 (20.7%)	0.60	45 (24.3%)	10 (31.2%)	0.41
LVMI*	23 (13.2%)	6 (20.7%)	0.29	20 (10.8%)	6 (18.8%)	0.20
sST2	**(n = 151)**	**(n = 85)**		**(n = 176)**	**(n = 60)**	
Time until echo, days	2 (1, 3)	2 (1, 3)	0.75	123 (118, 128)	122 (117, 126)	0.17
Metformin treatment	81 (53.6%)	42 (49.4%)	0.53	89 (50.6%)	34 (56.7%)	0.41
Septal e′ < 7 cm/s	53 (35.1%)	36 (42.4%)	0.27	50 (28.4%)	23 (38.3%)	0.15
Lateral e′ < 10 cm/s	80 (53.0%)	49 (57.6%)	0.49	75 (42.6%)	33 (55.0%)	0.096
E/e′ ratio > 14	4 (2.6%)	0 (0.0%)	0.13	2 (1.1%)	4 (6.7%)	0.019
GLS < 16%	50 (62%)	37 (76%)	0.11	54 (42.9%)	25 (58.1%)	0.083
LAVI > 34 ml/m^2	23 (18.4%)	12 (15.4%)	0.58	39 (24.4%)	16 (28.1%)	0.58
LVMI*	19 (15.2%)	10 (12.8%)	0.64	19 (11.9%)	7 (12.3%)	0.94

Table 2. Individual echocardiographic measurements of diastolic dysfunction during hospitalization, and at 4 months compared based on plasma levels of fibrotic markers. Septal e′, early diastolic tissue velocity from septal wall; Lateral e′, early diastolic tissue velocity from lateral wall; GLS, left ventricular global longitudinal strain; LAVI, left atrial volume indexed for body mass; LVMI, left ventricular mass indexed for body mass. * > 95 g/m^2 in women and > 115 g/m^2 in men.

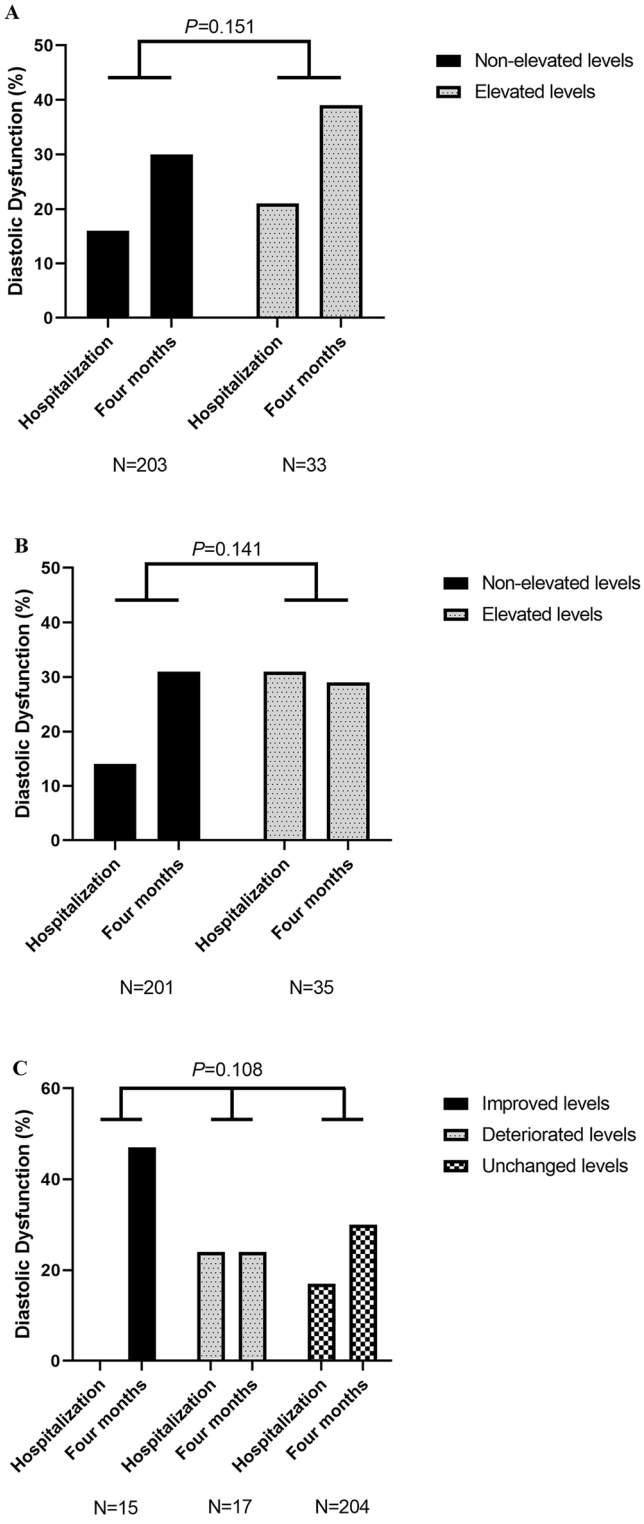

Figure 3. Association of diastolic dysfunction over time with elevated plasma levels of galectin-3 at hospitalization (**A**), 4 months follow-up (**B**), and categorical change between hospitalization and 4 months follow-up (**C**).

Elevated levels of sST2 have been associated with cardiac fibrosis, and its prognostic value is comparable to that of galectin-3 in heart failure patients. A previous study reported that ST2 correlated weakly with biomarkers of acute injury but was strongly associated with the risk of heart failure after myocardial infarction[30].

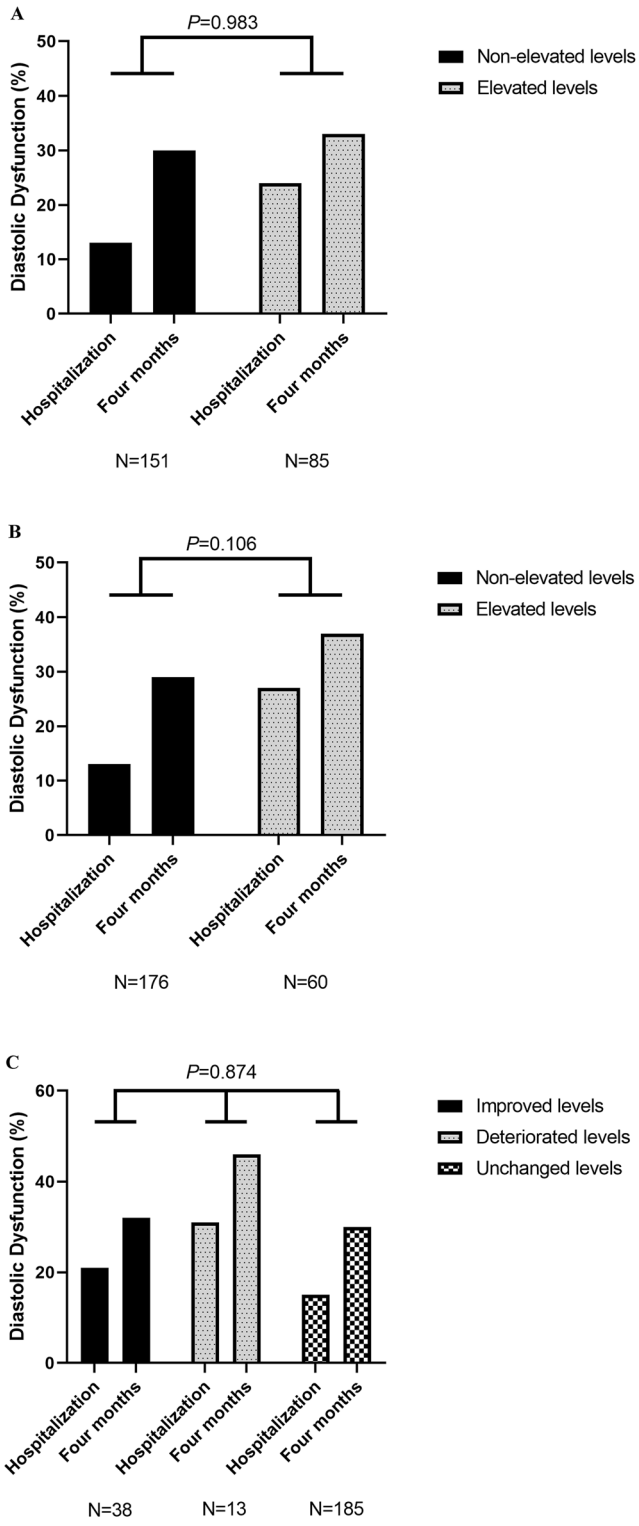

Figure 4. Association of diastolic dysfunction over time with elevated plasma levels of sST2 at hospitalization (**A**), 4 months follow-up (**B**), and categorical change between hospitalization and 4 months follow-up (**C**).

In a previous post-hoc analyses of the same study we evaluated the association of both biomarkers with systolic function post-STEMI. We observed that patients with elevated galectin-3 levels at the time of acute MI have lower systolic function after 4 months. However, in the current study we did not find a similar association with diastolic function. We did find that plasma levels of the fibrotic biomarkers of patients with a first STEMI were relatively stable over time when comparing plasma levels at presentation with the plasma levels at 4 months follow-up. Similarly, a study on kinetics of selected serum markers of fibrosis in patients with dilated

cardiomyopathy found that galectin-3 levels remained stable over 12 months regardless of diastolic dysfunction grade[31]. However, the pathophysiological chronology of dilated cardiomyopathy does not necessarily correspond to that of a STEMI. In murine models of post-myocardial infarction, galectin-3 rises directly after myocardial infarction both on tissue level and in the plasma level. However, it declines after a few days/weeks to nearly normal[32]. This corresponds with the timing of the scar formation. Exploring the predictive potential of maximum galectin-3 levels or the area under the curve of galectin-3 during the post-STEMI period warrants further investigation. A more detailed understanding of galectin-3 dynamics in post-STEMI patients may offer valuable insights into its clinical relevance.

Assessing diastolic dysfunction has proven to be much more difficult with definitions continuously being altered. It would be beneficial to know whether biomarkers could help us predict which patients are at risk to develop diastolic dysfunction after myocardial infarction. However, in this study, we could not identify patients that could be classified as at risk for the development of diastolic dysfunction by utilizing fibrotic markers alone. The latter could be due to the complexity of ventricular stiffness of the myocardium, which could not be represented by one single biomarker. A previous study in patients with hypertrophic cardiomyopathy showed that the combined parameters of NT-proBNP and maximum wall thickness had a better diagnostic performance for myocardial fibrosis assessed by late gadolinium enhancement on cardiovascular magnetic resonance imaging[33]. Moreover, ventricular stiffness is thought to be comprised of more than just interstitial fibrosis. Paulus et al. previously proposed a paradigm where a systemic proinflammatory state induced by comorbidities results in stiff cardiomyocytes and interstitial fibrosis. These alterations both contribute to a high diastolic left ventricular stiffness and heart failure development[34]. Additionally, diastolic dysfunction encompasses more than ventricular stiffness, reflecting the unique myocardial function of active relaxation[35], which fibrotic markers may not fully capture. The diversity of underlying mechanisms and variables that influence diastolic dysfunction is further underlined by a recent study that identified a distinct profile of five microRNA's that were significantly regulated in heart failure patients with preserved ejection fraction compared to healthy controls. These microRNA's have previously been shown to be involved in the regulation of endothelial function and cellular ageing. The same study found that the SGLT2 inhibitor empagliflozin improves the regulation of two of these microRNA's linked to endothelial function, indicating a possible reversibility[36].

In this study we did not find evidence that biomarkers of fibrosis by themselves can predict the development of diastolic dysfunction after STEMI. However, results from a previous study show that fibrosis remains a promising target for therapy. Schimmel et al. identified two novel antifibrotic therapeutics based on naturally derived substance library screens for the treatment of cardiac fibrosis. These substances were shown to be effective antifibrotic molecules both in vitro and in vivo, leading to improvement in diastolic function in two hypertension-dependent rodent models of cardiac fibrosis[37].

Galectin-3 and sST2 are recognized as independent predictors of adverse events and mortality in patients with heart failure[38–40]. However, our study did not reveal an association with long term outcome of MACE. One reason for this could be the fact that we have a low prevalence of symptomatic heart failure in our cohort. Additionally, while we maintained a substantial follow-up period of five years, the incidence of events was comparatively low. Consequently, our capacity to thoroughly investigate clinical outcomes is constrained by these factors.

Study limitations

A major strength of our study is that diastolic function was a predefined secondary endpoint in the prospective, randomized, placebo controlled GIPS-III trial. However, there are also some limitations in our study.

Echocardiographic assessment of diastolic dysfunction remains a continuing matter of debate and definitions differ between guidelines, and over time. Our study defined diastolic dysfunction based on the HFA-PEFF score which incorporates multiple criteria including tricuspid regurgitation peak velocity. Unfortunately, this parameter was not available in our dataset. As a results of this absent data our study is susceptible to non-differential misclassification bias. This bias may have led to individuals with diastolic dysfunction being misclassified as having a normal diastolic function, potentially attenuating associations with fibrosis related biomarkers. Based on the individual HFA-PEFF scores we were able to identify four (2%) patients with a potential for misclassification of diastolic dysfunction at hospitalization and 34 (14%) patients with a potential for misclassification of diastolic dysfunction at follow-up.

Roughly a third of the original cohort was excluded because echocardiography did not allow for diastolic measurements or because echocardiography was not performed. While we did not find evidence of a selection bias, we cannot exclude that an unknown patient selection based on presence of echocardiographic measurements has influenced our results.

Our study found no association between fibrosis related biomarkers and the development of diastolic dysfunction post-STEMI. However, these post-hoc analyses might be underpowered to detect small differences.

Conclusion

In summary, galectin-3 and sST2 were not associated with the development of diastolic dysfunction in non-diabetic patients that presented with a STEMI.

Data availability

The dataset analyzed during the current study is available from the corresponding author on reasonable request.

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Competing interests

The authors declare no competing interests.

Additional information

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