Exploring myocardial fibrosis in severe aortic stenosis: echo, CMR and histology data from FIB-AS study

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Abstract

Purpose

Myocardial fibrosis in aortic stenosis (AS) is associated with worse survival following aortic valve replacement (AVR). We assessed myocardial fibrosis in severe AS patients, integrating echocardiographic, cardiovascular magnetic resonance (CMR) and histological data.

Methods

A total of 83 severe AS patients (age 66.4 ± 8.3, 42% male) who were scheduled for surgical AVR underwent CMR with late gadolinium enhancement (LGE) and T1 mapping and global longitudinal strain (GLS) analysis. Collagen volume fraction (CVF) was measured in myocardial biopsies (71) that were sampled at the time of AVR.

Results

CVF correlated with imaging and serum biomarkers of LV systolic dysfunction and left side chamber enlargement and was higher in the sub-endocardium compared with midmyocardium ($p<0.001$). CVF median values were higher in LGE-positive versus LGE-negative patients [28.7% (19-33) vs 20.7% (15-30), respectively, $p=0.040$]. GLS was associated with invasively (CVF; $r=-0.303$, $p=0.013$) and non-invasively (native T1; $r=-0.321$, $p<0.05$) measured myocardial fibrosis. GLS and native T1 correlated with parameters of adverse LV remodelling, systolic and diastolic dysfunction and serum biomarkers of heart failure and myocardial injury.

Conclusion

Our data highlight the role of myocardial fibrosis in adverse cardiac remodelling in AS. GLS has potential as a surrogate marker of myocardial fibrosis, and high native T1 and low GLS values differentiated patients with more advanced cardiac remodelling.

Introduction

Myocardial fibrosis is fundamental in the pathogenesis of heart failure in the spectrum of cardiovascular diseases [1]. It is associated with the disruption of normal myocardial structure by excessive deposition of the extracellular matrix and creates a mechanistic base for adverse cardiac remodelling [2]. Myocardial fibrosis in aortic stenosis (AS) patients has been linked to impaired left ventricular (LV) function and adverse clinical outcomes [3].

Changes in cellular and extracellular matrix architecture, triggered by the greater afterload and wall stress in AS, increases tissue stiffness and impairs contraction [4, 5]. This complex interplay between components of cardiac remodelling can be evaluated by histological analysis of myocardial biopsy samples or the use of advanced imaging techniques with ability of tissue characterization.

Cardiovascular magnetic resonance (CMR), strengthened by the development of T1 mapping, provides a non-invasive and global estimation of myocardial fibrosis. Two distinct types of myocardial fibrosis can be depicted by CMR: the late gadolinium enhancement (LGE) technique quantifies focal fibrosis [6, 7], and diffuse interstitial expansion can be measured by T1 mapping [8]. Multicentre trials and meta-analyses have shown that the presence and extent of LGE are predictors of worse survival following aortic valve replacement (AVR), indicating advanced myocardial injury [9, 10]. Focal myocardial fibrosis is also irreversible following AVR [11, 12], effecting incomplete recovery of LV function and worse post-operative clinical outcomes, suggesting delayed timing of aortic valve intervention in some patients.

Several studies in AS patients have reported that native T1 and extracellular volume (ECV) values correlate with the degree of diffuse myocardial fibrosis, predict cardiovascular events and mortality [13–15] and are reversible with afterload relief [16], demonstrating potential as an early marker of adverse remodelling.

As a possible surrogate marker of myocardial fibrosis, LV myocardial global longitudinal strain (GLS), as assessed by speckle tracking echocardiography (STE), has been shown to be an independent predictor of adverse events in patients with severe AS, both with preserved and impaired LV systolic function [17].

Thus, novel diagnostic strategies and more accurate evaluations of the disease severity and consequences of AS are needed in assessing subclinical myocardial dysfunction to further risk-stratify severe AS patients. There are limited studies on myocardial fibrosis that have integrated multimodality imaging and sufficient histological analyses in severe AS. The optimal T1 image analysis strategy remains debated, requiring further validation. Our prospective study aims to: (i) non-invasively assess markers of myocardial fibrosis and validate them against histological data in patients who are undergoing surgical AVR and (ii) identify early imaging biomarkers of adverse LV remodelling in severe AS patients.

Methods

Study population and protocol

In this prospective observational study at Vilnius University Hospital between November 2018 and December 2020, patients with severe symptomatic AS that were scheduled for AVR according to current treatment recommendations [18] were recruited. The study was approved by the Biomedical Research Ethics Committee of the Vilnius Region (Approval Number: 158200-18/9-1014-558) and was performed as part of the FIB-AS study (NCT03585933). This study conformed to the principles of the Helsinki Declaration, and all subjects gave written consent to participate.

Patients were recruited prior to a pre-operative assessment and underwent a clinical assessment, comprising a clinical history, the Minnesota Living with Heart Failure Questionnaire (MLHFQ), the 6-minute walking test (6MWT), blood sampling [for haematocrit, renal function, brain natriuretic peptide (BNP) and high sensitivity troponin I (Hs-Tn-I)], a transthoracic echocardiogram, and CMR. The inclusion

criteria were patients who were undergoing AVR for severe AS [defined as aortic valve area (AVA) ≤1 cm^2 or AVA index ≤0.6 cm^2/m^2, as determined by ultrasonography], age >18 years, ability to undergo a CMR scan, and consent to the study protocol. The exclusion criteria were significant coronary artery disease (CAD) (>50% lesion), history of myocardial infarction, severe valve disease other than AS, estimated glomerular filtration rate <30 mL/min/1.73 m^2, CMR-incompatible devices, persistent atrial tachyarrhythmias, and previous cardiac surgery (Fig. 1). The study data were collected and stored in a dedicated online database, REDCap (Research Electronic Data Capture) [19].

Fig. 1 FIB-AS study flow chart

Cardiac Imaging

ECHOCARDIOGRAPHY- Transthoracic 2D echocardiography was performed using a commercially available Vivid ultrasound system (S70, E9 or E95) (GE Healthcare, Horten, Norway), and the data were stored on a dedicated workstation for subsequent off-line analysis. AS severity and LV systolic and diastolic function were evaluated per the echocardiographic guidelines [20, 21]. AVA was calculated using the continuity equation.

2D SPECKLE TRACKING ECHOCARDIOGRAPHY (STE)- From the 2D grey-scale images of the apical 2-, 3- and 4-chamber views, LV global longitudinal strain (GLS) was measured and processed off-line using commercially available software (EchoPac 112.0.1, GE Medical Systems, Horten, Norway) [22]. The frame rate was adjusted to 50 to 80 frames/s. End-systole was defined, based on the closure click on the spectral tracing of the pulsed-wave Doppler of AV flow. GLS was acquired using the average regional strain curves (16-segment model for 2D STE). Segments with poor quality tracking or aberrant curves (despite manual adjustment) were removed from analysis. Due to missing data or poor image quality, STE analysis was completed for 77 of 83 patients.

CMR PROTOCOL- CMR scans were obtained using standard protocols on a 1.5 T Siemens Aera scanner with surface coils and prospective electrocardiography (ECG) triggering. LV end-systolic and end-diastolic diameters and maximum wall thickness were traced and recorded from the short-axis and long-axis views of the standard ECG-gated steady-state-free precession cine sequence. LV volumes, mass and ejection fraction were measured using commercial software (suiteHEART®) from a stack of sequential 8-mm short-axis slices (0-2-mm gap) from the atrio-ventricular ring to the apex. Measurements were indexed to body surface area in m^2 (using the DuBois formula).

LGE IMAGING- To detect delayed hyperenhancement, images were acquired 10–15 min after intravenous administration of gadobutrol (0.2 mmol/kg) (Gadovist, Bayer AG, Germany) using a breath-hold segmented inversion recovery fast-gradient echo sequence in the short-axis and long-axis planes of the LV, with an 8-mm slice thickness and 20% distance factor. The region of myocardial fibrosis was defined as the sum of pixels with a signal intensity above 5 standard deviations of the normal remote

myocardium in each short-axis slice. The presence of LGE was determined qualitatively by two independent readers who were blinded to the clinical data.

T1 MAPPING- T1 mapping images were acquired in 4-chamber long-axis and short-axis (at the midventricular levels) images before and 15 min after contrast administration. All T1 mapping images were acquired using the modified Look-Locker inversion-recovery sequence [23, 24] with the Motion Correction technique. T1 maps were generated from the CMR workstation after in-line motion correction just after image acquisition. Regions of interest were drawn manually in the blood and septum at the midventricular level on the short-axis image, excluding the myocardium with LGE. The ECV of the myocardium was calculated as follows: ECV% = (ΔR1m/ΔR1b) x (1 - haematocrit level) x 100, where R1 is 1/T1, R1m is R1 in the myocardium, R1b is R1 in the blood and ΔR1 is the change in relaxation [25]. Due to incomplete datasets, T1 mapping parameters were measured in 67 of 83 patients.

Histological Analysis

At the time of surgical AVR, biopsy specimens were obtained under direct vision by the surgical team using a surgical scalpel from the basal anteroseptum just after removal of the diseased AV. One intraoperative myocardial biopsy sample (mean area 22.5 ± 12 mm^2) was taken from each patient. All myocardial tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (3-µm-thick) were sliced on a Leica RM2145 microtome and stained with haematoxylin and eosin and Masson's trichrome. Digital images were captured by on an Aperio Scan-Scope XT Slide Scanner (Aperio Technologies, Vista, CA, USA) under 20× objective magnification (0.5 µm resolution). Histologists who were blinded to the clinical and CMR data examined all biopsy specimens.

The fraction of myocardial volume that was occupied by collagen tissue (collagen volume fraction, CVF) was determined by quantitative morphometry on an automated image analysis system (HALO™). The area of myocardial fibrosis was calculated using the HALO™ Area Quantification v2.1.11 algorithm (IndicaLabs, NM, USA) [26]. The subendocardial layer was defined as 1 mm from the endocardial surface, whereas the rest of the tissue sample was defined as the midmyocardial layer.

Statistical analysis

Variables were presented as mean ± standard deviation or median and interquartile ranges. Categorical variables were expressed as frequencies and percentages and were compared by v2 test. Unpaired student's t-test and Mann–Whitney U test were used to compare two groups of continuous variables. Pearson's and Spearman's correlation coefficients were calculated to assess the relationships between continuous variables.

Intra- and inter-observer variation was analysed by Bland-Altman method and calculation of the correlation coefficient. The statistical analysis was performed in R (version 3.5.1). Differences were considered statistically significant provided a 2-sided p value < 0.05 [27].

Results

A total of 83 patients were included (age 66.4 ± 8.3 years, 58% female, AVA index 0.44 ± 0.1 cm^2/m^2, peak AV velocity 4.8 ± 0.6 m/s, mean gradient 57.8 ± 16 mm Hg). The main reasons for non-eligibility were significant CAD, renal dysfunction and other valvular abnormalities. The mean LV ejection fraction (LVEF) was 66.8 ± 13%, with 10% of patients having reduced LVEF (<50%). Overall, patients had low surgical risk, with STS-PROM and EuroScore < 4% (1.9% and 1.5%, respectively). Patients with congenital AS were more likely to be younger (p<0.001), were at lower surgical risk (p=0.004), and had better renal function (p=0.002). Of the 83 enrolees, 79 underwent surgical AVR and 4 postponed surgery due to Covid-19. The patients' clinical and imaging characteristics are summarised in Tables 1 and 2.

Table 1
Patients clinical characteristics

Variable	All patients (n=83)
Age, yrs	66.4 ± 8.3
Male gender	35 (42%)
BMI, kg/ m^2	30 ± 5.8
BSA, m^2	1.9 ± 0.2
Systolic BP, mmHg	150 ± 25
Diastolic BP, mmHg	85 ± 11
Comorbidities	
Hypertension	73 (90%)
Dyslipidemia	66 (82%)
Unobstructive CAD	39 (48%)
Diabetes mellitus	14 (17%)
Atrial fibrillation	6 (7%)
History of PCI	1 (1%)
Symptoms and functional status	
Dyspnea	60 (74%)
Chest pain	39 (48%)
Syncope	9 (11%)
NYHA functional class	
I	16 (19%)
II	24 (29%)
III	40 (48%)

Continuous variables are presented as mean ± SD or median [interquartile range]. Categorical variables are expressed as n (%).

6 MWT, 6 minutes walking test; BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; BSA, body surface area; CAD, coronary artery disease; ECG, electrocardiography; MLHFQ, Minnesota living with heart failure questionnaire; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; S-L, Sokolow Lyon voltage criterion; STS, Society of Thoracic Surgeons' risk model score; EuroScoreII, European System for Cardiac Operative Risk Evaluation II score; ACE-I, angiotensin-converting-enzyme inhibitor; ARB, angiotensin-receptor blocker; hs-Tn-I, high sensitivity troponin I; eGFR, estimated glomerular filtration rate

Variable	All patients (n=83)
IV	3 (4%)
6 MWT, m	357.6 ± 105.6
MLHFQ score	35 ± 20.4
Drug history	
ACE-I/ARB	60 (74%)
Betablocker	56 (69%)
Statin	53 (65%)
Loop diuretic	15 (19%)
Spironolactone	22 (27%)
Risk scores	
STS-PROM, %	1.9 (1.2-2.3)
EuroSCORE II, %	1.5 (0.7-1.6)
Surgery	
Tissue valve	89%
Mechanical valve	11%
Aortic intervention	4%
Valve morphology	
Tricuspid	52 (64%)
Bicuspid	28 (35%)
Unicuspid	1 (1%)
Blood tests	
Creatinine µmol/l	76.2 ± 16.3

Continuous variables are presented as mean ± SD or median [interquartile range]. Categorical variables are expressed as n (%).

6 MWT, 6 minutes walking test; BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; BSA, body surface area; CAD, coronary artery disease; ECG, electrocardiography; MLHFQ, Minnesota living with heart failure questionnaire; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; S-L, Sokolow Lyon voltage criterion; STS, Society of Thoracic Surgeons' risk model score; EuroScoreII, European System for Cardiac Operative Risk Evaluation II score; ACE-I, angiotensin-converting-enzyme inhibitor; ARB, angiotensin-receptor blocker; hs-Tn-I, high sensitivity troponin I; eGFR, estimated glomerular filtration rate

Variable	All patients (n=83)
eGFR, ml/min/1.73 m^2	78.6 (69- 90)
Hs-Tn-I, pg/l	116.5 (5 - 18.7)
BNP, pg/l	374.6 (65.2- 339.6)
ECG parameters	
Heart rate, beats/min	77 ± 12.4
S-L criteria (mm)	30.8 ± 10
QRS duration, ms	96.8 (88- 102)

Continuous variables are presented as mean ± SD or median [interquartile range]. Categorical variables are expressed as n (%).

6 MWT, 6 minutes walking test; BMI, body mass index; BNP, brain natriuretic peptide; BP, blood pressure; BSA, body surface area; CAD, coronary artery disease; ECG, electrocardiography; MLHFQ, Minnesota living with heart failure questionnaire; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; S-L, Sokolow Lyon voltage criterion; STS, Society of Thoracic Surgeons' risk model score; EuroScoreII, European System for Cardiac Operative Risk Evaluation II score; ACE-I, angiotensin-converting-enzyme inhibitor; ARB, angiotensin-receptor blocker; hs-Tn-I, high sensitivity troponin I; eGFR, estimated glomerular filtration rate

Table 2
Patients imaging characteristics.

Echocardiography data	
Peak AV velocity, m/s	4.8 ± 0.6
Mean AV gradient, mm Hg	57.8 ± 16
Low gradient AS	10 (12%)
AVA, cm^2	0.84 ± 0.2
AVA index, cm^2/ m^2	0.44 ± 0.1
IVSd, mm	12.7 ± 1.7
Posterior wall diameter, mm	11.5 ± 1.4
LVdd, mm	51.4 ± 5.4
LVsd, mm	32.7 ± 5.9
E/A	1.1 ± 0.5
E deceleration time, ms	259 ± 70
E/e' septal	17.6 ± 7
E/e' lateral	14.5 ± 6
E/e' mean	15.6 ± 6
LA volume index, ml/ m^2	47.9 ± 12
PASP, mm Hg	38 ± 15
RV S', cm/s	11.6 ± 3
TAPSE	21.7 ± 3
GLS, %	-18 ± 5
CMR and histology data	
Continuous variables are presented as mean ± SD or median [interquartile range]. Categorical variables are expressed as n (%).	
AV, aortic valve; AVA, aortic valve area; E, peak early velocity of the transmitral flow; CMR, cardiovascular magnetic resonance; CVF, collagen volume fraction; *e'*, peak early diastolic velocity of the mitral annulus displacement; GLS, global longitudinal strain; ECV, extracellular volume; IVSd, interventricular septum diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; LA, left atrium; LGE, late gadolinium enhancement; PASP, pulmonary artery systolic pressure measured by echocardiography; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; RVESV, right ventricular end-systolic volume; RV S', peak systolic velocity of the tricuspid annulus displacement; TAPSE, tricuspid annulus plane systolic excursion	

Echocardiography data	
IVSd, mm	13.3 ± 2
LVdd, mm	50.6 ± 6
LVsd, mm	33.8 ± 8
LVEDV, ml	144.3 ± 44
LVESV, ml	51 (27.9- 60.7)
LV stroke volume index, ml/m^2	48 ± 11
LVEF, %	66.8 ± 13
LVEF < 50%	12 (10%)
LV mass index, g/m^2	97.6 ± 32
RVEDV, ml	125.3 ± 31
RVESV, ml	49.3 ± 18
RVEF, %	60.8 ± 10
Native T1, ms	959.7 ± 34
Post-contrast T1, ms	351 (326- 362)
ECV, %	22.7 ± 3.6
T2, ms	41 (40- 44)
Patients with LGE	61 (74%)
No of LGE segments per patient	2.5
CVF, %	15.1 (9-21)
CVF subendocardial, %	21.1 (12-29)

Continuous variables are presented as mean ± SD or median [interquartile range]. Categorical variables are expressed as n (%).

AV, aortic valve; AVA, aortic valve area; E, peak early velocity of the transmitral flow; CMR, cardiovascular magnetic resonance; CVF, collagen volume fraction; e', peak early diastolic velocity of the mitral annulus displacement; GLS, global longitudinal strain; ECV, extracellular volume; IVSd, interventricular septum diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; LA, left atrium; LGE, late gadolinium enhancement; PASP, pulmonary artery systolic pressure measured by echocardiography; RVEDV, right ventricular end-diastolic volume; RVEF, right ventricular ejection fraction; RVESV, right ventricular end-systolic volume; RV S', peak systolic velocity of the tricuspid annulus displacement; TAPSE, tricuspid annulus plane systolic excursion

Myocardial Fibrosis By Histology

Of 71 myocardial biopsies, 2 were epicardial. One myocardial biopsy was excluded from the analysis due to an incidental finding of toxoplasmic myocarditis. The median CVF was 15.1% (8.6-21). Patients with higher CVF had a greater prevalence of hypertension (p=0.024) and dyslipidaemia (p=0.036). Higher values of CVF were observed in LGE-positive versus LGE-negative patients—28.7% (19-33) vs 20.7% (15-30), respectively (p=0.040). No significant differences in median CVF value were noted between patients with and without CAD [17.2% (10-23) vs 13.4% (9-19), respectively; p=0.094]. Segmental analysis of myocardial biopsies revealed more fibrosis in the subendocardial layer compared with a midmyocardial layer [21.1% (12-29) vs 8% (5-12); p< 0.001; Fig. 2).

Fig. 2 Image on the left shows myocardial biopsy sample stained with Masson‘s trichrome. Graph on the right shows comparison of collagen volume fraction (CVF) in different layers of myocardium. Higher proportion of collagen detected in subendocardium compared to midmyocardium

Myocardial Fibrosis By Cmr

The median delay between CMR and surgery was 53.3 days (17-78). Mean native T1 was 959.7 ± 34 ms (range: 897–1044 ms), and the mean ECV was 22.7 ± 3.6% (range: 15.7% - 34.4%). No significant difference in mean native T1 and ECV values was observed between men and women (962 ± 29 ms vs 957 ± 37 ms, p=0.391 and 22.9 ± 3% vs. 22.6 ± 4%, p=0.821, respectively).

To compare native T1 with clinical and structural parameters, we divided variables (above and below the median: 957 ms, Table 3). Patients with elevated native T1 had lower systolic blood pressure (p=0.006), higher QRS voltage on the ECG (p=0.036), greater systolic (p=0.009) and diastolic LV dimensions (p=0.049) and higher LV mass index (p=0.021). Among those with elevated native T1, a higher proportion of patients had reduced GLS (18% vs 6%, respectively; p=0.049).

Table 3
Patients clinical and imaging characteristics stratified by median GLS and native T1 values

	GLS ≤ 18.5% (n=40)	GLS >18.5% (n=37)	P-value	Native T1 ≥ 957 ms (n=34)	Native T1 < 957 ms (n=33)	P-value
Age, yrs	66 ± 8	68 ± 8	0.256	65.8 ± 9	66 ± 9	0.917
Male gender	18 (45%)	14 (38%)	0.548	15 (44%)	11 (33%)	0.446
BSA, m^2	1.98 ± 0.2	1.86 ± 0.2	**0.004**	1.96 ± 0.16	1.93 ± 0.19	0.607
Systolic BP, mmHg	143 ± 23	158 ± 23	**0.005**	139 ± 21	156 ± 26	**0.006**
Diastolic BP, mmHg	83 ± 11	85 ± 11	0.485	82 ± 10	86 ± 13	0.203
Unobstructive CAD	20 (50%)	18 (49%)	1.0	20 (59%)	14 (42%)	0.893
Hypertension	36 (90%)	33 (89%)	0.447	27 (79%)	33 (100%)	0.109
Diabetes mellitus	8 (20%)	4 (11%)	0.768	6 (18%)	7 (21%)	1.0
NYHA f.cl. ≥ 3	26 (65%)	14 (38%)	0.085	16 (47%)	15 (46%)	0.749
MLHFQ score	37 ± 20	32 ± 20	0.257	37 ± 21	36 ± 20	0.839
6 MWT, m	351 ± 105	358 ± 104	0.767	367 ± 106	352 ± 94	0.558
ECG						
HR, b/min	80 ± 14	75 ± 11	0.100	78 ± 4	77 ± 12	0.742
QRS, ms	95 (90-102)	90 (86-98)	0.105	94 (89-102)	90 (84-101)	0.313
S-L, mm	34 ± 11	28 ± 8.5	**0.011**	34 ± 10	29 ± 9	**0.036**
Echo data						
AVA index, cm^2/m^2	0.42 ± 0.1	0.47 ± 0.08	**0.018**	0.4 ± 0.1	0.45 ± 0.1	0.075
Peak AV velocity, m/s	5.0 ± 0.7	4.7 ± 0.5	0.055	5.0 ±0.6	4.8 ± 0.6	0.105
Mean gradient, mmHg	63 ± 17.7	53 ± 13.2	**0.004**	64 ± 16	57 ± 15	0.052
IVSd, mm	13.3 ± 1.8	12.2 ± 1.4	**0.009**	13 ± 1.9	12.6 ± 1.6	0.368
LVdd, mm	53.7 ± 12	48.8 ± 4.7	**0.002**	53 ± 5	50 ± 5	**0.049**

Continuous variables are presented as mean ± SD or median [interquartile range]. Categorical variables are expressed as n (%). The boldface values indicate statistical significance. Abbreviations as in Tables 1 and 2

	GLS ≤ 18.5% (n=40)	GLS >18.5% (n=37)	P-value	Native T1 ≥ 957 ms (n=34)	Native T1 < 957 ms (n=33)	P-value
LVsd, mm	35.4 ± 6	29.6 ± 4	**<0.001**	35 ± 6	32 ± 6	0.057
E deceleration time, ms	254 ± 76	264 ± 67	0.542	252 ±68	266 ± 75	0.759
E/e' septal	17.1 (14-22)	14 (11.7-18)	**0.011**	16.5 (12.8-18)	16 (12-20)	0.845
E/e' mean	17.4 ± 6.9	14.2 ± 4.4	**0.021**	15 ± 5	16 ± 7	0.909
LA volume index, ml/m^2	53 ± 12	44 ± 11	**0.002**	48 ± 9	48 ± 15	0.473
PASP, mmHg	43.5 ± 18	32.9 ± 7	**0.031**	41 ± 17	37 ± 12	0.947
GLS,%	14.3 ± 3.9	21.7 ± 2.7	**<0.001**	16.7 ± 5.6	18.2 ± 4	0.120
GLS >-15%	16 (40%)	-	**<0.001**	10 (29%)	4 (12%)	**0.049**
CMR and histology data						
IVSd, mm	14 ± 2	12.6 ± 2	**0.005**	14 ± 1.6	13 ± 2.3	0.364
LVdd, mm	53 ± 7	48.3 ± 5	**<0.001**	52 ± 6	50 ± 5	0.074
LVsd, mm	37 ± 9	30.6 ± 6	**<0.001**	36.5 ± 7	32 ± 6	**0.009**
LVEDV, ml	160.7 ± 48	126 ± 35	**<0.001**	153 ± 40	143 ± 44	0.201
LVESV, ml	56.9 (41-77)	29 (24-41)	**<0.001**	52 (37-72)	41 (28-53)	0.083
LVEF, %	59 ± 14	74 ± 7	**<0.001**	62.4 ± 14	68 ± 12	0.053
LVEF <50%	8 (20%)	0	**0.009**	6 (18%)	2 (6%)	0.541
LV mass index, g/m^2	113 ± 33	80.6 ± 24	**<0.001**	109 ± 31	91 ± 30	**0.021**
LGE prevalence	34 (85%)	23 (62%)	0.058	27 (79%)	25 (76%)	0.802
Native T1, ms	967 ± 31	950 ± 37	0.066	987 ± 26	936 ± 18	**<0.001**
Post-contrast T1, ms	349 (326-354)	355 (332-366)	0.201	352 (328-362)	348 (318-362)	0.445
ECV, %	22.3 ± 4	22.9 ± 2.4	0.456	23 ± 3.2	22 ± 3.9	0.243

Continuous variables are presented as mean ± SD or median [interquartile range]. Categorical variables are expressed as n (%). The boldface values indicate statistical significance. Abbreviations as in Tables 1 and 2

	GLS ≤ 18.5% (n=40)	GLS >18.5% (n=37)	P-value	Native T1 ≥ 957 ms (n=34)	Native T1 < 957 ms (n=33)	P-value
T2, ms	43 (41-45)	42 (40-44)	0.196	43.3(41-45)	42(40-44)	0.291
BNP, pg/l	252 (98-813)	79 (59-173)	**0.001**	163 (73-581)	120 (62-260)	0.413
Hs-Tn-I, pg/l	15 (7.5-29)	6.9 (5-12.9)	**0.002**	14 (7-27)	7.5 (5-16)	0.089
CVF, %	17.2 (10-22)	13.5 (8-20)	0.279	18.1 (8-24)	13.4 (10-21)	0.564
CVF subendocardial,%	23.4 (13-33)	18.4 (11-27)	0.199	22.3 (9-28)	18.8 (12-26)	0.855

Continuous variables are presented as mean ± SD or median [interquartile range]. Categorical variables are expressed as n (%). The boldface values indicate statistical significance. Abbreviations as in Tables 1 and 2

Focal fibrosis, measured by LGE, was common, affecting 74% of all patients (83% of men and 67% of women). Further, 92% of focal fibrosis was the non-infarct type (89% mid-myocardial, 3% subepicardial). Despite having unobstructed coronary arteries 8% of patients had infarct-type focal fibrosis. The most common location of LGE was the right ventricular insertion point (68%). LGE was also detected in the anterolateral (11%), septal (8%), posterolateral (6%), inferior (6%) and apical (1%) segments. We found no significant difference in the prevalence of LGE between patients with and without CAD (77% and 70%, respectively; p=0.67). Compared with patients without focal fibrosis, LGE-positive subjects had more severe AS, as evidenced by smaller AVA index (p=0.02), thicker LV walls (p<0.001), higher LV mass index (p=0.01) and larger left atrial (LA) volume index (p=0.03). Patients with LGE also had higher levels of BNP (p=0.004) and Hs-Tn-I (p=0.003).

Longitudinal Deformation Analysis

The mean GLS was -18 ± 5% (range: -3% to -31%), and a reduction in GLS of less than 20% was observed in 61% of patients.

To analyse GLS with regard to clinical and structural parameters, we dichotomised the variables (above and below median: -18.5%; Table 3). Patients with lower GLS had more severe AS, based on smaller AVA index (p=0.018), higher mean transvalvular gradient (p=0.004), lower systolic blood pressure (p=0.005) and greater QRS voltage on the ECG (p=0.011). The low-GLS group also had thicker LV walls (p=0.009), higher LV volumes (p<0.001), greater LV mass index (p<0.001) and lower LVEF (p<0.001). This group showed signs of elevated LV filling pressures, as evident by higher E/e' ratios (p=0.011), with consequently higher LA volume index (p=0.002) and pulmonary artery systolic pressure (p=0.031). Higher levels of BNP (p=0.001) and Hs-Tn-I (p=0.002) were detected in these patients. Representative images of

patients with various degrees of LV remodelling by echocardiography, CMR and histology are shown in Fig. 3.

Fig. 3 Four exemplar patients showing progressive cardiac remodeling: continuous-wave Doppler (maximum velocities >4 m/s; Column 1), global longitudinal strain (GLS; Column 2), short axis cine stills demonstrating degrees of left ventricular (LV) remodeling (Column 3), matching native T1 (Column 4) and collagen volume fraction (CVF) in myocardial biopsies stained with Masson's trichrome (Column 5). Patient A has preserved GLS, minimal LV hypertrophy, low native T1 and CVF of 11.6%. Patient B has reduced GLS, concentric LV hypertrophy, higher native T1 and moderate histological fibrosis (CVF-17.6%). Patient C has low GLS, evidence of LV hypertrophy, high native T1 and significant histological fibrosis (CVF-23.5%). Patient D, with decompensated heart failure, has low GLS, LV cavity dilatation, high native T1 and extensive histological fibrosis (CVF- 40.8%)

Analysis Of Associations

CVF correlated with LV end-diastolic diameter (r=0.242, p=0.043), LV end-systolic volume (r=0.265, p=0.028), LVEF (r=-0.246, p=0.04) and LA volume index (r=0.314, p=0.009). When subendocardium was excluded from the analysis, CVF correlated with LV mass (r=0.247, p=0.041), LVEF (r=-0.354, p=0.003), GLS (r=-0.303, p=0.013) and BNP (r=0.242, p=0.045) (Fig. 4).

Fig. 4 Correlations between histological myocardial fibrosis (CVF) and LV ejection fraction (a), LV mass (b), GLS (c) and brain natriuretic peptide (BNP) (d) are shown. Abbrevations are as in Figure 3.

With regards to LV structure and function, GLS correlated with LV end-diastolic volume (r=-0.485, p<0.001), LV end-systolic volume (r=-0.636, p<0.001), LV mass index (r=-0.615, p<0.001) and LVEF (r=0.7, p<001). GLS was also linked to parameters that were associated with elevated LV filling pressures: mean E/e' (r=-0.4, p=0.002), LA volume index (r=-0.405, p<0.001) and estimated pulmonary artery systolic pressure (r=-0.376, p<0.05). Native T1 correlated with LV end-systolic volume (r=0.349, p=0.003), LV end-diastolic volume (r=0.269, p=0.03), LV mass index (r=0.414, p<0.001) and LVEF (r=0.317, p<0.05). GLS and native T1 were associated with the degree of AS severity: AV mean gradient (r=-0.387, p<0.001 and r=0.408, p<0.001, respectively) and AVA (r=0.30, p<0.05 and r=0.3, p=0.02, respectively).

With regard to serum biomarkers, GLS and native T1 correlated with BNP (r=-0.653, p<0.001 and r=0.371, p<0.05, respectively) and hs-Tn-I (r=-0.486, p<0.001 and r=0.333, p<0.05, respectively) and with each other (r=-0.321, p<0.05)(Fig. 5).

Fig. 5 Correlations between GLS and native T1 (a), GLS and BNP (b), native T1 and BNP (c) are shown. Abbrevations are as in Figure 4.

Reproducibility Of Measurements

The intraclass correlation coefficients for native T1 were 0.945 (95% CI 0.88–0.97, bias 3.3 ± 11.0 ms) for intra-observer variation and 0.958 (95% CI 0.91–0.98, bias 9.1 ± 15.1 ms) for inter-observer variation. The GLS measurements also demonstrated excellent reproducibility: 0.969 (95% CI 0.93–0.98, bias 0.51 ± 1.3) for intra-observer variation and 0.981 (95% CI 0.96–0.99, bias 1.5 ± 1) for inter-observer variation.

Discussion

This prospective study presents a comprehensive assessment of the consequences of AS on LV myocardium by integrating CMR and STE data with a large number of myocardial biopsies.

The main study findings are as follows:

1) The non-infarct type of focal fibrosis is highly prevalent in severe low-risk AS patients who are free of significant CAD.

2) Histologically measured myocardial fibrosis is associated with imaging and serum biomarkers of LV systolic dysfunction and left side chamber enlargement.

3} The subendocardium is affected by myocardial fibrosis to a greater extent and determines longitudinal dysfunction.

4) GLS is associated with invasively and non-invasively measured myocardial fibrosis; low GLS and elevated native T1 differentiated patients with more advanced LV remodelling.

Compared with previous studies in severe AS patients, our cohort was younger and free from significant CAD, thus representing low-risk isolated AS patients. Although 90% of our study population had preserved LVEF, a more detailed assessment of myocardial structure and function through cardiac imaging and histological analysis revealed evidence of varying degrees of myocardial injury.

The amount of fibrosis in the myocardial biopsies varied substantially, from 2–41%. Diffuse fibrosis, which is present in healthy myocardium, constituted less than 2%, based on the autopsy results of subjects who died of non-cardiovascular causes [28, 29]. If the amount of myocardial fibrosis increases with age is less clear. We found that histological myocardial fibrosis was associated with LV and LA enlargement and worse systolic function, underscoring the role of myocardial fibrosis in the pathophysiological progression to cardiac decompensation in AS, in terms morphology and function. Consistent with earlier studies, we found that the subendocardial layer contained more fibrosis compared with a midmyocardium. Gradients of myocardial fibrosis in the LV wall have been described in patients with severe AS and those with hypertrophic cardiomyopathy and hypertensive heart disease—conditions that are both associated with chronic pressure overload and an increase in LV mass [28, 29]. These findings can be explained by a transmural gradient of wall stress and ischemia in the subendocardial layer due to the relative decrease in capillary density, with subsequent cell loss and reparative fibrosis [30].

GLS and native T1 median values differentiated patients with more advanced LV remodelling, wherein patients with lower GLS and higher native T1 had evidence of altered LV structure, diastolic and systolic impairments and higher levels of serum biomarkers, indicative of heart failure and myocardial injury. Notably, patients with reduced GLS and elevated native T1 still had preserved LVEF, and only 20% of patients with adverse structural and functional cardiac remodelling had LVEF below 50%. Thus, only 1 in 5 patients with advanced cardiac remodelling can be detected if only this echocardiographic criterion of cardiac decompensation is used, overlooking a substantial number of patients who would benefit from early AV intervention. Our results are consistent with previous studies, showing that fibrotic changes that are induced by AS begin in the subendocardium and initially affect longitudinal function, which is not well represented by LVEF, because it can be compensated by global radial function [7, 31].

Notably, patient groups did not differ by symptom status, functional capacity or quality of life assessment. This finding suggests that symptom assessments can be challenging and misleading and do not always reflect true cardiac condition, indicating that the decision to intervene should be supported by objective markers of cardiac injury, rather than based on subjective assessment of symptom status.

Imaging biomarkers, or the integration of several parameters, might be particularly useful in patients with no or minimal symptoms or when ascertaining valve-related symptoms is challenging. Our data implicate GLS and native T1 as early markers of cardiac decompensation. GLS can also be used as a surrogate marker of myocardial fibrosis, as it was associated with both, invasively and non-invasively measured myocardial fibrosis.

Seventy-four percent of our patients had areas of focal fibrosis, 98% of which were the non-infarct type and which were independent of the presence of nonobstructive CAD. Although only 1 or 2 segments were affected by LGE in most patients, data from a recent large multicentre study show that >2% of LGE in patients with severe AS who undergo AVR is associated with worse postoperative survival [32]. We found, that the myocardium of patients who have progressed to more advanced myocardial injury and have developed areas of irreversible replacement fibrosis on CMR also contains higher degree of diffuse fibrosis measured histologically. Unexpectedly, we found no associations between CVF and CMR markers of diffuse fibrosis, for which there are several explanations. There was a possible sampling error, because only 1 biopsy sample per patient was analysed. Further, the histological and T1 mapping analyses were performed at different levels and layers of the interventricular septum. The myocardial biopsies were endocardial and taken from the basal anteroseptum, possibly containing higher amounts of fibrotic tissue, and the region of interest for the T1 mapping measurements was drawn in the middle of the septum at the midventricular level, avoiding endo and epicardial borders. The data in this field are inconsistent, with some studies reporting significant associations between invasively and non-invasively measured myocardial fibrosis in AS cohorts [15, 33] and others failing to demonstrate this association [24, 29].

Although our patients presented with increased LV mass and myocardial fibrosis in the histological analysis, ECV values were not elevated in our cohort compared with our local reference range. This

finding can be explained by the greater increase in cellular mass (adaptive hypertrophy), as opposed to the expansion of extracellular space, because ECV per se represents the percentage of space that is occupied by the extracellular compartment of the total LV mass. The average native T1 and ECV values in our cohort were lower in comparison to AS populations in other studies [13, 15]. A large T1 mapping data variability across different centers have been previously reported, influenced by differences in field strength, vendor-specific set-up and variations in sequences [34, 35]. Disparities in ECV values can also be expected with the non-uniformity of contrast agents and their doses [36]. Another explanation for such variability relates to differences in the study cohorts. When interpreting our results, we should consider that we examined relatively young, low-risk patients who were free from significant CAD, whereas other studies, especially those that included transcatheter treatment cohorts, enrolled patients who were in their 80s and had a higher rate of comorbidities [37].

Conclusion

A comprehensive assessment of LV response to AS by integrating histology, CMR and STE reveals varying degrees of myocardial injury that are not apparent with traditional measures of LV systolic function. Histological myocardial fibrosis was associated with imaging and serum biomarkers of LV systolic dysfunction and left side chamber enlargement. We found that native T1 by CMR and GLS by STE differentiated patients with advanced cardiac remodelling, constituting a marker of subclinical cardiac damage. Of all imaging parameters, only GLS was associated with invasively and non-invasively measured myocardial fibrosis, demonstrating its potential as a surrogate marker of myocardial fibrosis.

Study Limitations

The study was composed of a small number of AS patients, however it included substantial number of myocardial biopsies. Due to the Covid-19 pandemic, delays in patient examinations and surgeries were experienced, causing uneven time frames between the preoperative patient assessment (echocardiographic and CMR) and surgery with myocardial sampling, potentially affecting the final result. Proportion of histologically measured myocardial fibrosis could have been affected by the size and depth of biopsy samples, as more superficially sampled and smaller biopsies may contain higher proportion of fibrotic tissue in comparison to larger biopsy samples. Although measuring T1 values only in the septum is a validated and common method, it might not represent the entire myocardium. Because we excluded patients with comorbidities, such as obstructive CAD, a history of myocardial infarction, renal failure and persistent atrial arrhythmias, our results should not be overgeneralized to the broader AS patient population.

Abbreviations

6MWT
6-minute walking test
AS

aortic stenosis
AV
aortic valve
AVR
aortic valve replacement
BNP
brain natriuretic peptide
CAD
coronary artery disease
CMR
cardiovascular magnetic resonance
ECG
electrocardiography
ECV
extracellular volume
GLS
global longitudinal strain
Hs-Tn-I
high-sensitivity troponin I
LA
left atrium
LGE
late gadolinium enhancement
LV
left ventricle
LVEF
left ventricular ejection fraction
MLHFQ
Minnesota Living With Heart Failure Questionnaire
NYHA
New York Heart Association
STE
speckle tracking echocardiography

Declarations

Funding

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Competing interests

The authors declare that they have no competing interests.

Author contributions

Sigita Glaveckaitė and Peter Sogaard are chief investigators; they conceived the study, led the proposal and protocol development. Giedrė Balčiūnaitė drafted the manuscript. Pranas Šerpytis, Audrius Aidietis and Tomas Zaremba contributed to study implementation. Nomeda Valevičienė and Darius Palionis performed CMR scanning and data analysis. Kęstutis Rušinskas, Vilius Janušauskas and Aleksejus Zorinas performed aortic valve replacement surgeries and sampled myocardial biopsies. Edvardas Žurauskas and Justinas Besusparis performed histological analysis. Viktor Skorniakov conceived and developed the statistical aspects of the study. All authors reviewed and approved the final version of the manuscript.

Ethics approval

The study conformed to the principles of the Helsinki Declaration, and all subjects gave written consent to participate.The study (protocol, including qualitative and quantitative aspects, and trial materials, including patient information and consent form) was reviewed and approved by the Biomedical Research Ethics Committee of the Vilnius Region (16/March/2018; No: 158200-18/9-1014-558).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The authors affirm that human research participants provided informed consent for publication of the images in Figure 3.

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Figures

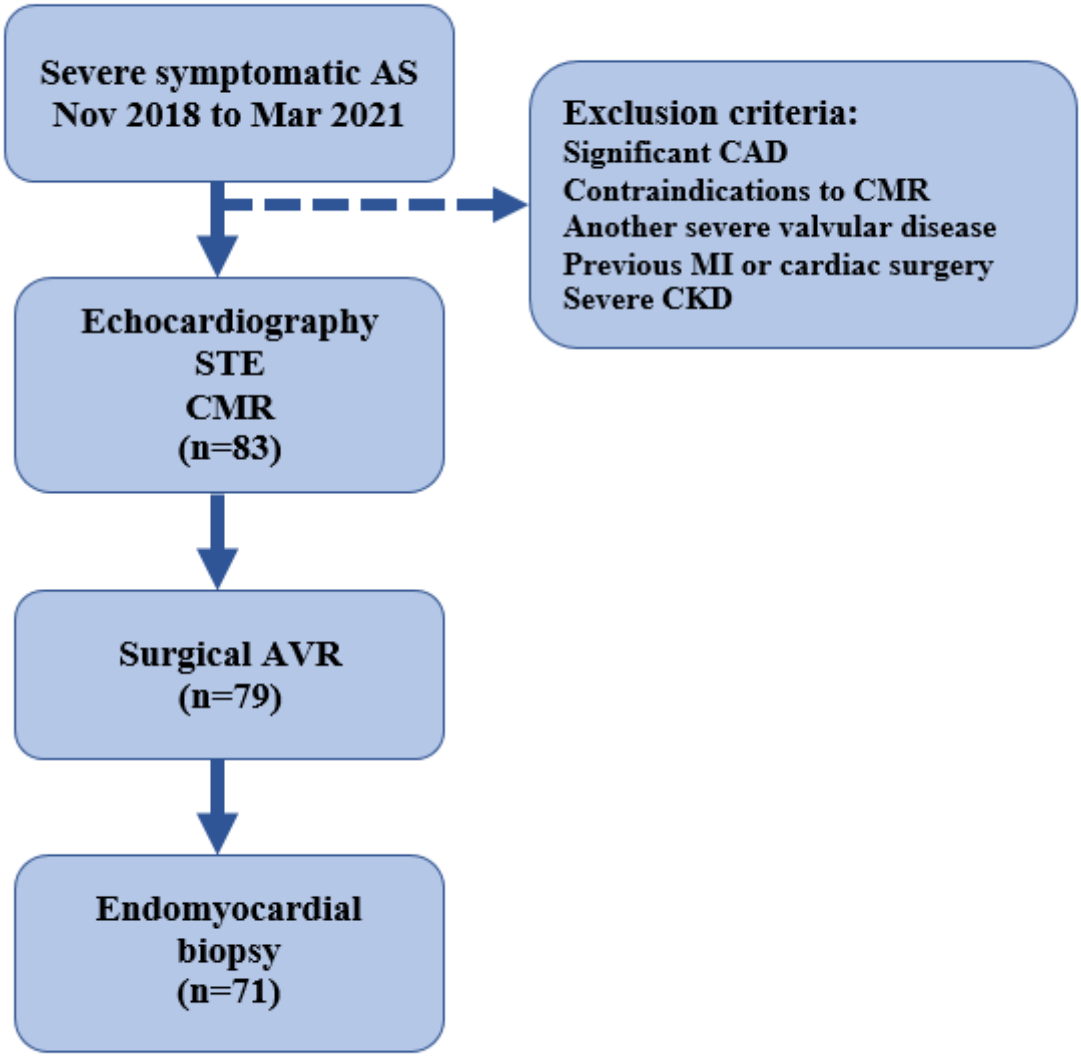

Figure 1

FIB-AS study flow chart

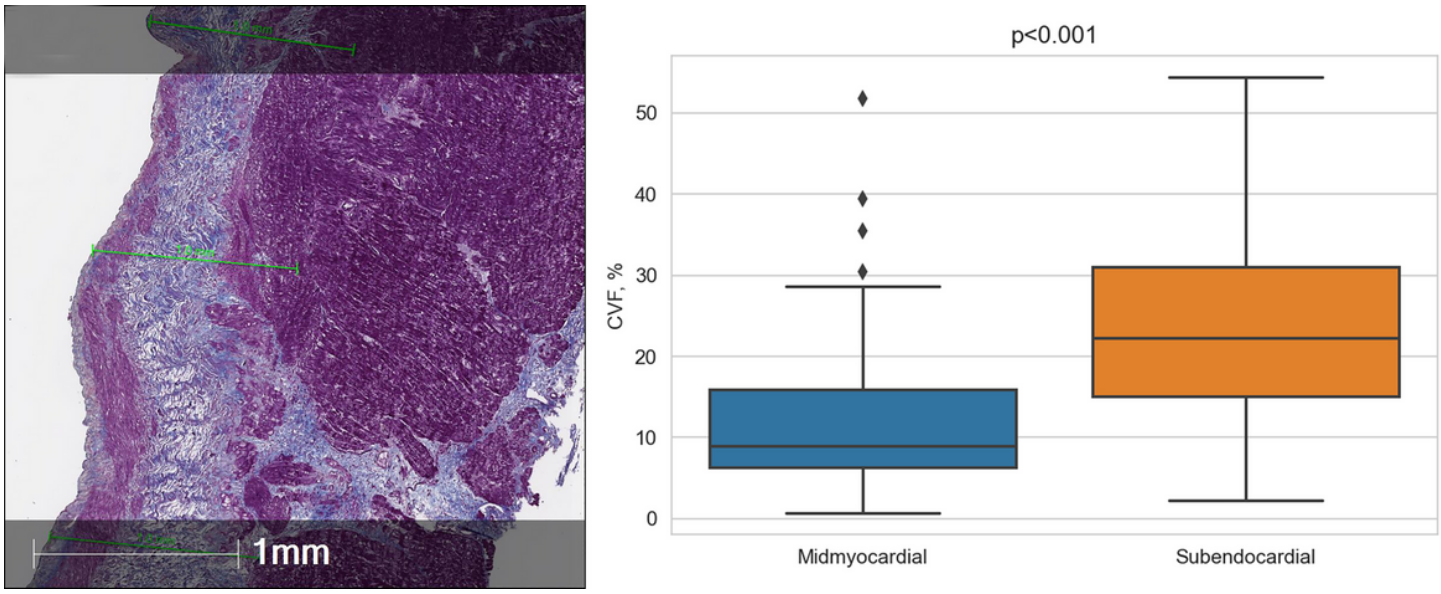

Figure 2

Image on the left shows myocardial biopsy sample stained with Masson‘s trichrome. Graph on the right shows comparison of collagen volume fraction (CVF) in different layers of myocardium. Higher proportion of collagen detected in subendocardium compared to midmyocardium

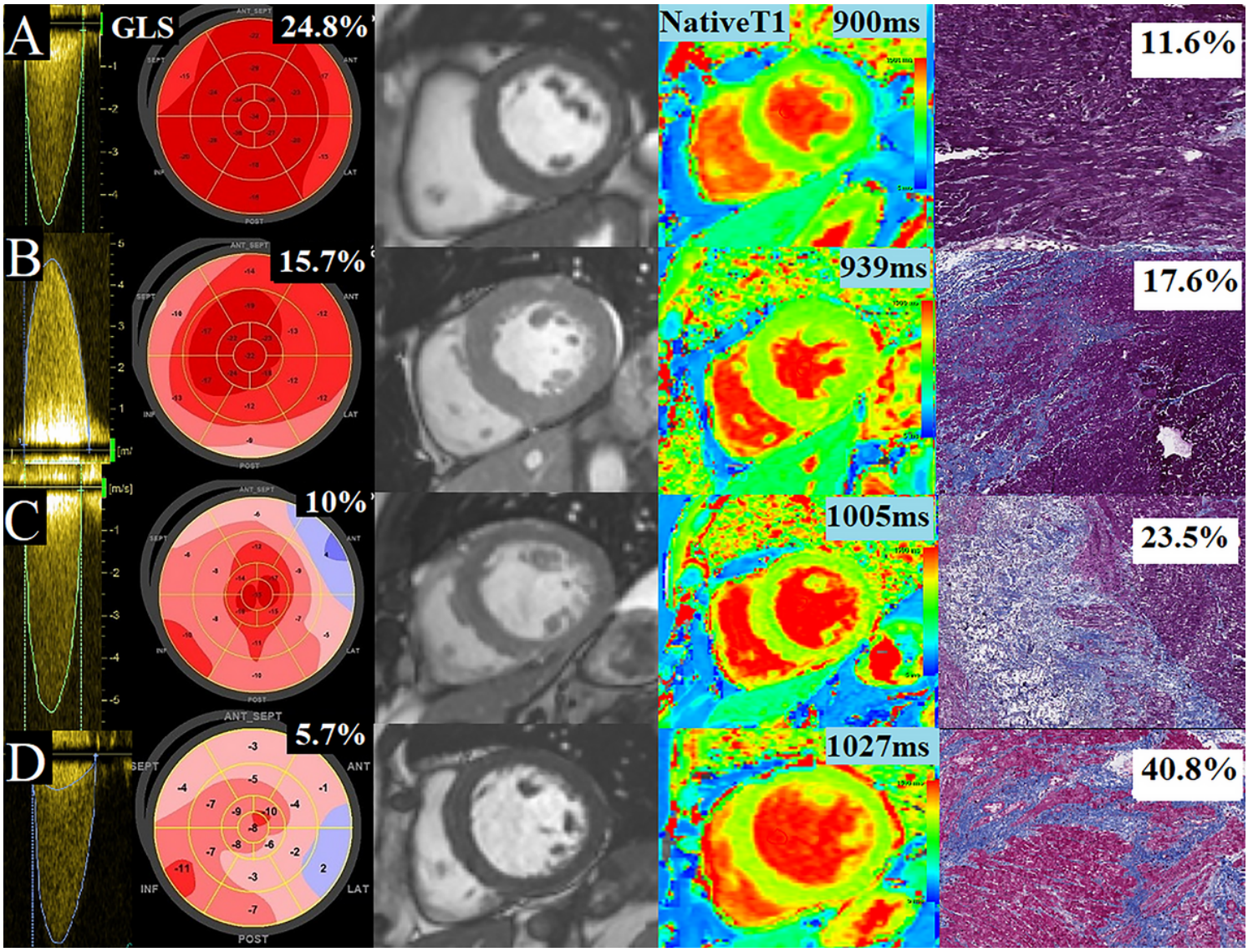

Figure 3

Four exemplar patients showing progressive cardiac remodeling: continuous-wave Doppler (maximum velocities >4 m/s; Column 1), global longitudinal strain (GLS; Column 2), short axis cine stills demonstrating degrees of left ventricular (LV) remodeling (Column 3), matching native T1 (Column 4) and collagen volume fraction (CVF) in myocardial biopsies stained with Masson's trichrome (Column 5). Patient A has preserved GLS, minimal LV hypertrophy, low native T1 and CVF of 11.6 %. Patient B has reduced GLS, concentric LV hypertrophy, higher native T1 and moderate histological fibrosis (CVF-17.6 %). Patient C has low GLS, evidence of LV hypertrophy, high native T1 and significant histological fibrosis (CVF-23.5%). Patient D, with decompensated heart failure, has low GLS, LV cavity dilatation, high native T1 and extensive histological fibrosis (CVF- 40.8%)

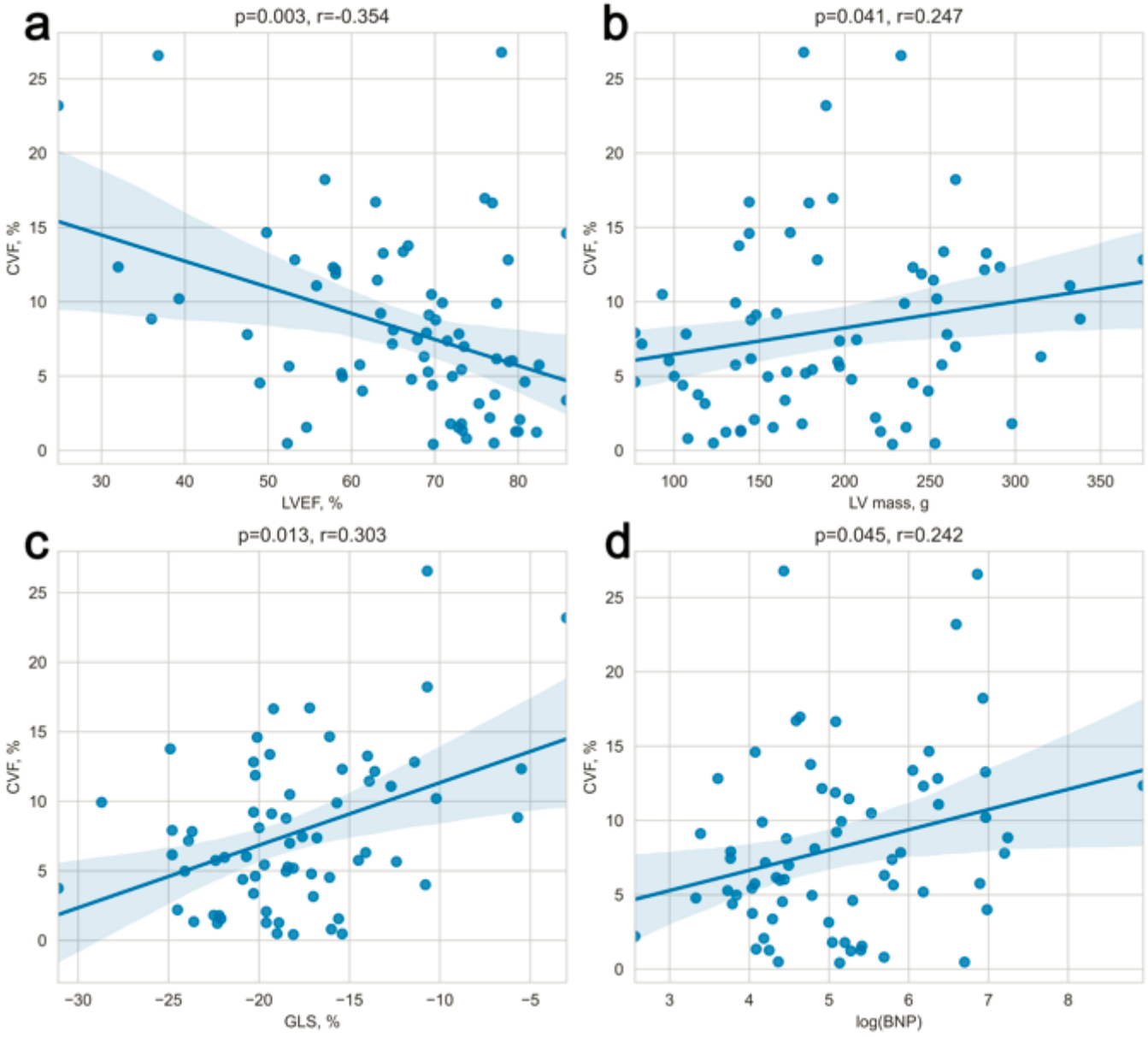

Figure 4

Correlations between histological myocardial fibrosis (CVF) and LV ejection fraction (a), LV mass (b), GLS (c) and brain natriuretic peptide (BNP) (d) are shown. Abbrevations are as in Figure 3.

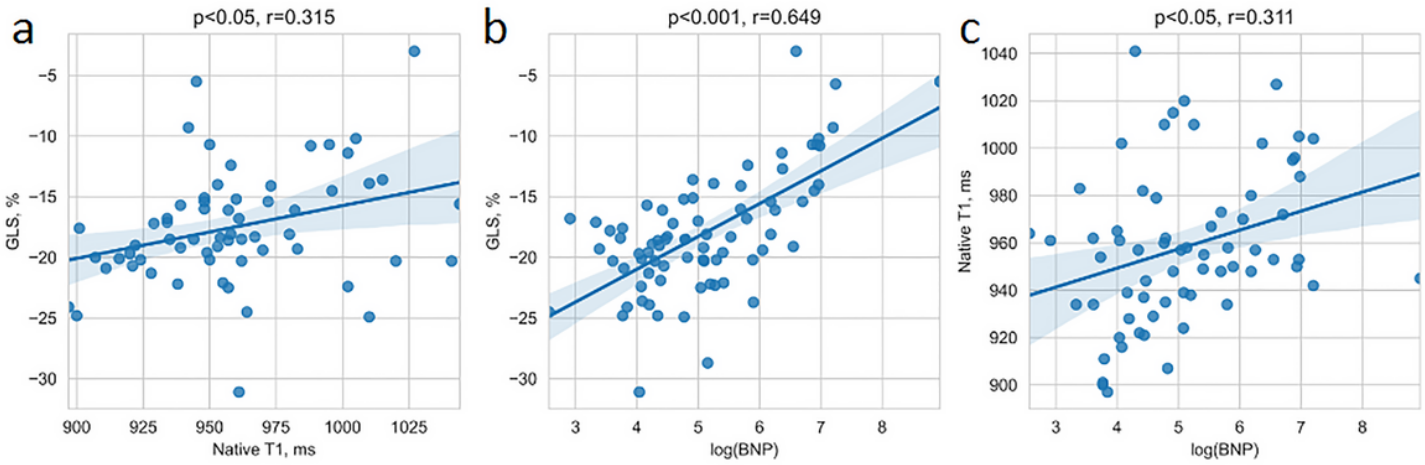

Figure 5

Correlations between GLS and native T1 (a), GLS and BNP (b), native T1 and BNP (c) are shown. Abbrevations are as in Figure 4.