Reappraising myocardial fibrosis in severe aortic stenosis: an invasive and non-invasive study in 133 patients

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Aims
To investigate myocardial fibrosis (MF) in a large series of severe aortic stenosis (AS) patients using invasive biopsy and non-invasive imaging.

Methods and results
One hundred thirty-three patients with severe, symptomatic AS accepted for surgical aortic valve replacement underwent cardiovascular magnetic resonance (CMR) with late gadolinium enhancement (LGE) and extracellular volume fraction (ECV) quantification. Intra-operative left ventricular (LV) biopsies were performed by needle or scalpel, yielding tissue with (n = 53) and without endocardium (n = 80), and compared with 10 controls. Myocardial fibrosis occurred in three patterns: (i) thickened endocardium with a fibrotic layer; (ii) microscopic scars, with a subendomyocardial predominance; and (iii) diffuse interstitial fibrosis. Collagen volume fraction (CVF) was elevated (P < 0.001) compared with controls, and higher (P < 0.001) in endocardium-containing samples with a decreasing CVF gradient from the subendocardium (P = 0.001). Late gadolinium enhancement correlated with CVF (P < 0.001) but not ECV. Both LGE and ECV correlated independently (P < 0.001) with N-terminal pro-brain natriuretic peptide and high-sensitivity-troponin T. High ECV was also associated with worse LV remodelling, left ventricular ejection fraction and functional capacity. Combining high ECV and LGE better identified patients with more adverse LV remodelling, blood biomarkers and histological parameters, and worse functional capacity than each parameter alone.

Conclusion
Myocardial fibrosis in severe AS is complex, but three main patterns exist: endocardial fibrosis, microscars (mainly in the subendomyocardium), and diffuse interstitial fibrosis. Neither histological CVF nor the CMR parameters ECV and LGE capture fibrosis in its totality. A combined, multi-parametric approach with ECV and LGE allows best stratification of AS patients according to the response of the myocardial collagen matrix.

Keywords
Myocardial fibrosis • Aortic stenosis • Cardiovascular magnetic resonance • Late gadolinium enhancement • Extracellular volume fraction

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Introduction

In aortic stenosis (AS), patient symptoms and outcome are determined by the severity of the valve stenosis, but also by the myocardial response to the generated afterload—a process that appears crucial, but is incompletely understood.1 Our scientific exploration of this uses two main approaches. Clinically, the myocardium is measured by assessing structure and function using imaging (echocardiography, cardiovascular magnetic resonance (CMR)).2 Pathophysiological, the myocardium is assessed histologically on tissue samples. It is believed that a complex interplay of cellular changes (including hypertrophy and cell death by apoptosis or autophagy), microvascular ischaemia, and alterations of the extracellular matrix occurs with final common pathways leading to myocardial fibrosis (MF). Most of the evidence for this has been from a few small biopsy or autopsy studies. Whereas autopsy descriptions of MF can provide a global view, in vivo studies, sampling is limited by biopsy size, and fibrosis is typically described only by the quantity of collagen deposition [collagen volume fraction (CVF)]. However, histological analysis of heart tissue also allows differentiation of fibrosis subtypes based on location and morphological characteristics of collagen deposits (focal microscopic scars, diffuse interstitial and perivascular strands; see Supplementary material online, Figure S1), with the functional impact of MF not only depending on the amount of collagen tissue but also on the characteristics of collagen deposits.3 Although new insights are being generated by imaging tissue characterization [the late gadolinium enhancement (LGE) technique permits quantification of focal interstitial expansion,4–8 and diffuse interstitial expansion can be measured by extracellular volume fraction (ECV)], the histological basis of LGE and ECV in AS and their association with fibrosis subtypes are only partly understood.

We investigated myocardial fibrosis in a large series of symptomatic severe AS patients using invasive biopsy and non-invasive imaging. We simultaneously and at scale assessed cardiac status by measuring functional capacity and blood biomarkers (cardiomyocyte stress/damage markers), by imaging structure and function (echocardiography and CMR), and by performing non-invasive (ECV and LGE) and histological (fibrosis location, pattern, and CVF) tissue characterization.

Methods

Study cohort

A single centre, prospective observational cohort study at University College London Hospital NHS Trust between January 2012 and January 2015 of patients with severe, symptomatic AS undergoing surgical aortic valve replacement (AVR) with or without coronary artery bypass grafting (CABG) using invasive and non-invasive assessment. The study was approved by the ethical committee of UK National Research Ethics Service (07/H0715/101) and was performed as a planned sub-study of RELIEF-AS (ClinicalTrials.gov NCT02174471). The study conformed to the principles of the Helsinki Declaration, and all subjects gave written consent to participate. Patients were recruited prior to pre-operative assessment and underwent clinical assessment with clinical history, blood pressure, 6-minute walk test (6MWT), blood sampling (haematocrit, renal function, N-terminal pro-brain natriuretic peptide (NT-proBNP) and high sensitivity troponin T (hs-TnT)), transthoracic echocardiogram, and CMR.

Inclusion criteria: patients undergoing AVR ± CABG for severe AS (two or more of: AVA <1 cm², pressure gradient ≥40 mmHg (mean), velocity time integral (VTI) ratio <0.25 or reclassification of discordant echocardiographic data to severe by alternate modality); consenting for study protocol; age >18 years, ability to undergo CMR scan. Exclusion criteria: pregnancy/breastfeeding, estimated glomerular filtration rate <30 mL/min, CMR incompatible devices, previous valve surgery, infective endocarditis, severe valve disease other than AS or other planned concurrent valve operations (severe AS with mild or moderate AR was acceptable).

Control myocardial samples were obtained from autopsies of 10 subjects (7 male, 3 female; all Caucasian, age: 60 ± 7 years) who died of non-cardiovascular causes showing no signs of macroscopic or microscopic cardiac lesions.

Cardiac imaging

Echocardiography assessed diastolic function and valve area/velocities (with CMR for regurgitant volumes if needed). Cardiac magnetic resonance assessed structure, function and myocardial tissue characterization. Echocardiography used a GE Vivid E9 system (GE Healthcare, Wauwazota, USA) with a 4-MHz transducer as per current guidelines. Cardiovascular magnetic resonance was performed at 1.5 Tesla (Magnetom Avanto, Siemens Medical Solutions) with 32 channel cardiac coil arrays, using a standard clinical scan protocol with LGE imaging and T1 mapping prior to and after bolus gadolinium contrast (0.1 mmol/kg of Gadoterate meglumine (gadolinium-DOTA, marketed as Dotarem, Guerbet S.A., Paris, France)). Post-contrast imaging was performed at 10 min (LGE) and 15 min (T1 mapping). The T1 mapping sequence used was a balanced-SSFP-based MOdified Look-Locker Inversion Recovery (MOLLI) variants (investigational prototypes) with motion-correction (sampling scheme pre-contrast 5s(3s)3s and post-contrast 4s(1s)3s(1s)2s; system software version VB17).

Image analysis

Cardiovascular magnetic resonance imaging analysis was performed using CVH2 software (Version 5.1.2 [303], Calgary, Canada) blinded to clinical parameters. Left ventricular volume and mass analysis were performed by manual contouring of the endo- and epicardial borders at end-diastole and end-systole with papillary muscle and trabeculations included in the LV mass. Late gadolinium enhancement was quantified in grams and percentage of LV mass using a 3 standard deviations (SD) threshold. For T1 mapping, three short axis T1 maps (base, mid, and apex) were manually contoured for endo- and epicardial borders. Partial voluming of blood was minimized by an automatic 10% offset from the endo- and epicardial border (see Supplementary material online, Figure S2). Segments with infarct-pattern LGE (subendocardial LGE) were excluded from ECV analysis but non-infarct LGE was included (as per guidelines). Extracellular volume fraction was defined as ECV = (1-Hct) × [ΔR₁/myocardium]/[ΔR₁/blood].15 Normal ranges have been described previously.16

Histomorphological studies

Biopsies were harvested under direct vision from the basal anteroseptum when the native valve was removed by one of six surgeons using either a 14-gauge coaxial needle system (Temno evolution, Carefusion, USA) or a surgical scalpel, as per surgeon’s choice (as per ethics) and fixed in 10% buffered formalin and embedded in paraffin. Histological analysis was performed blinded to clinical and imaging data. For MF, the fraction of myocardial volume with positive staining for collagen, CVF, was determined by quantitative morphometry (CellID, Olympus Soft imaging Solutions GmbH, Münster, Germany) in sections stained with collagen-specific piercing red. All available myocardial tissue was analysed (average area was 5.21 ± 3.62 mm²/sample). Endocardial thickness was quantified as the mean value of 5–15 measurements.
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Table 1  Baseline characteristics

| Parameter                        | Total          |
|----------------------------------|----------------|
| n, male (%)                      | 133 (56%)      |
| Age (years)                      | 70.3 ± 9.6     |
| BMI (kg/m²)                      | 28.3 ± 5.1     |
| Co-morbidities                   |                |
| Hypertension                     | 101 (76%)      |
| SBP (mmHg)                       | 134 ± 18       |
| DBP (mmHg)                       | 76 ± 11        |
| Diabetes                         | 28 (21%)       |
| Coronary artery disease          | 45 (34%)       |
| Symptoms (yes/no)                | 127/6          |
| NYHA functional class I, II, III, IV | 26, 62, 41.4 |
| Chest pain                       | 43 (32%)       |
| Syncpe                           | 11 (8%)        |
| Six-minute walk test distance (m; median IQR) | 458 (318–572) |
| Risk scores                      |                |
| STS % (median IQR)               | 1.5 (1.0–2.4)  |
| EuroScoreII % (median IQR)       | 1.6 (1.0–2.5)  |
| Type of valve                    |                |
| Tricuspid                        | 95 (71%)       |
| Bicuspid                         | 37 (28%)       |
| Unicuspid                        | 1              |
| Echocardiography                 |                |
| Vmax (m/s)                       | 4.3 ± 0.6      |
| Peak gradient (mmHg)             | 75 ± 19        |
| Mean gradient (mmHg)             | 46 ± 13        |
| AVAi (cm²/m²)                    | 0.41 ± 0.13    |
| Diastolic function               |                |
| E-wave                           | 0.85 ± 0.29    |
| E deceleration time (ms)         | 237 ± 75       |
| E/e’ ratio                       | 13.7 ± 6.1     |
| PASP (mmHg)                      | 31 ± 8         |
| CMR parameters                   |                |
| EDVi (mL/m²)                     | 66 ± 22        |
| ESVi (mL/m²)                     | 22 ± 19        |
| LVM (g/m²)                       | 87 ± 24        |
| LVEF (%)                         | 70 ± 15        |
| SVi (mL/m²)                      | 44 ± 11        |
| CI (L/min/m²)                    | 3.2 ± 0.7      |
| Maximal wall thickness (mm)      | 14 ± 3         |
| LAAi (cm²/m²)                    | 13.5 ± 3.9     |
| CMR flow                         |                |
| Aortic regurgitant fraction in %, median IQR | 10.9 (3.3–24.3) |
| Mitral regurgitant fraction in %, median IQR | 4.0 (0–20.5) |
| Late gadolinium enhancement      |                |
| 3SD method in grams, median IQR | 10.5 (6.0–20.3) |
| T1 mapping (MOLLI)               |                |
| T1 myocardium (native in ms)     | 1043 ± 44      |
| ECV (%)                          | 28.4 ± 2.9     |
| Histology                        |                |
| Collagen volume fraction (%)     | 11.5 ± 8.6     |
| Endocardial thickness (µm)       | 228 ± 129      |

Table 1  Continued

| Parameter                        | Total          |
|----------------------------------|----------------|
| Drug history                     |                |
| ACE-I/ARB                        | 60 (45%)       |
| Betablocker                      | 45 (34%)       |
| Statin                           | 86 (65%)       |
| Aspirin                          | 54 (41%)       |
| Spironolactone                   | 3 (2%)         |
| Blood                            |                |
| NT-pro-BNP (pg/mL) (median IQR)  | 72 (29–242)    |
| hs-Troponin T (ng/L) (median IQR)| 13 (8–19)      |
| Creatinine (micromol/L)          | 85 ± 26        |
| eGFR (mL/min/1.73m²)             | 77 ± 22        |
| Haematocrit (%)                  | 40.0 ± 4.2     |

BSA, body surface area; SBP, systolic blood pressure; DBP, diastolic blood pressure; NYHA, New York Heart Association; IQR, interquartile range; STS, Society of Thoracic Surgeons’ risk model score; EuroScore II, European System for Cardiac Operative Risk Evaluation II score; Vmax, peak velocity through the aortic valve; AVAi, aortic valve area index; E, peak early velocity of the transmural flow; E, peak early diastolic velocity of the mitral annulus displacement; PASP, pulmonary artery systolic pressure measured by echocardiography; EDVi, end-diastolic volume index; ESVi, end-systolic volume index; LVMi, left ventricular mass index; LVEF, left ventricular ejection fraction; SVi, stroke volume index; CI, cardiac output indexed; LAAi, left atrial area index; 3SD, three standard deviations; ECV, extracellular volume; ACE-I, angiotensin-converting-enzyme inhibitor; ARB, angiotensin-receptor blocker; NT-proBNP, N-terminal pro-brain natriuretic peptide; hs-TnT, high sensitivity troponin T; eGFR, estimated glomerular filtration rate; CMR, cardiovascular magnetic resonance.

Statistical analysis

Statistical analyses used SPSS 22 (IBM, Armonk, NY, USA). Continuous variables were expressed as mean ± SD, categorical as percentages. Normality was checked using the Shapiro–Wilk test. Groups were compared using independent-samples t-test (if normal) or the Mann–Whitney U (if non-normal), and the χ² test for binomial variables. Correlations were estimated by using the Pearson correlation coefficient once normality was demonstrated; otherwise, the Spearman correlation coefficient, Log transformation was applied to normalize NT-proBNP and hs-TnT. The influence of potential confounding factors [age, gender, history of coronary artery disease (CAD)] used multivariate linear regression analysis. The unstandardized coefficient B and its 95% confidence interval were recorded. A two-sided P-value of <0.05 was considered significant.

Results

Baseline characteristics

One hundred and forty-four patients with severe, symptomatic AS underwent CMR prior to and myocardial biopsy at the time of AVR. Eleven patients were excluded due to inability to complete CMR [claustrophobia (n = 2), haemodynamic instability (n = 1)], incomplete CMR dataset (n = 1), or significant bystander disease known to affect LV remodelling and outcome (cardiac amyloidosis n = 6; Fabry Disease n = 1).17 One hundred thirty-three patients were included (age 70 ± 10 years, 56% male, AVAi 0.41 ± 0.13 cm²/m², Vmax 4.3 ± 0.6 m/s, mean gradient 46 ± 13 mmHg); all but 6 patients were symptomatic (96%) with dyspnoea (80%), chest pain (32%), and/or syncope (8%). Aetiology of AS was predominantly tricuspid (71%) with the...
remainder bicuspid (28%) or unicuspid AS ($n = 1$). The treatment received was tissue or mechanical valve replacement in 71% and 29%, respectively, with additional bypass grafting in 23% and aortic intervention in 6%. Baseline characteristics are shown in Table 1. Representative images of the AS severity by echocardiography (continuous-wave Doppler), LV remodelling (SSFP-cine short axis), and MF by CMR (LGE and ECV) and histology (CVF) are shown in Figure 1.

**Non-invasive assessment by cardiovascular magnetic resonance**

Focal fibrosis, measured by LGE, was commonly seen, affecting 71% of men and 46% of women, with a similar split in infarct-like pattern vs. non-infarct pattern LGE (males 16% vs. 59%; females 17% vs. 37%—some had both). The location of non-infarct LGE was right ventricular insertion point (60%), patchy focal (26%), papillary muscle (19%), and/or mid-myocardial (18%) (see Supplementary material online, Figure S2). Mean enhanced LV myocardial mass was 14.3 ± 11.2 g (median 10.5 g; interquartile range 6.0–20.3 g). Mean ECV was 28.4 ± 2.9%. Imaging findings are summarized in Table 1.

**Invasive assessment by biopsy**

Collagen volume fraction was elevated in severe AS (11.5 ± 8.6% vs. 1.95 ± 0.20% controls, $P < 0.001$) and was higher in men than in women (12.9 ± 8.8 vs. 9.9 ± 8.0%, $P = 0.030$). There were 53 myocardial biopsies with endocardium (mostly from scalpel biopsies, 60%) and 80 samples with no identifiable endocardium. Biopsies with...
endocardium showed higher CVF than biopsies without endocardium (15.0 ± 12% vs. 8.99 ± 6.7%; \( P < 0.001 \); Figure 2). The endocardium was thickened in AS patients due to collagen deposition in most biopsies, with a mean endocardial thickness of 228 ± 129 microns vs. 40 ± 16 microns in the control samples (\( P < 0.001 \); Figure 3A). Segmental analysis in tertiles of endocardium-containing biopsies (in those structurally feasible; \( n = 40 \)) revealed a decreasing gradient of fibrosis from the subendocardium towards the mid-myocardium (20.4 ± 11.3% vs. 15.2 ± 8.7% vs. 13.0 ± 7.8%, \( P \) for trend = 0.001; Figure 2). Of note, subendocardial fibrosis was caused predominantly by microscars, whereas mid-myocardial fibrosis was due to interstitial bands preferentially located around cardiomyocytes (Figure 3B).

**Analysis of associations**

Late gadolinium enhancement quantification correlated with CVF in all samples (\( r^2 = 0.248, P < 0.001 \)), but this association was stronger in endocardial containing samples (\( r^2 = 0.501, P < 0.001 \); Figure 4). These associations were independent of age, gender and history of CAD.
Collagen volume fraction quantification was weakly associated with NT-proBNP \( (r^2 = 0.055, \ P = 0.013) \) and hs-TnT \( (r^2 = 0.072, \ P < 0.01) \) levels in all patients. The correlation between CVF and NT-proBNP improved slightly when we considered only the endocardial samples \( (r^2 = 0.123, \ P = 0.027) \). However, these associations were lost when adjusting for confounding factors.

With regards to LV structure and function, both LGE and ECV correlated weakly with LV end-diastolic volume index (LVEDVi; \( r^2 = 0.038, \ P = 0.026 \) and \( r^2 = 0.066, \ P = 0.006 \), respectively), LV end-systolic volume index (LVESVi; \( r^2 = 0.067, \ P = 0.003 \) and \( r^2 = 0.114, \ P < 0.001 \), respectively) and left ventricular ejection fraction (LVEF) \( (r^2 = -0.055, \ P = 0.007 \) and \( r^2 = -0.096, \ P = 0.001 \), respectively); but the associations were only independent of confounding factors for ECV. Late gadolinium enhancement was weakly but independently correlated with LVMi \( (r^2 = 0.087, \ P = 0.001) \); ECV was not \( (P = 0.06) \).

With regards to biomarkers, both LGE and ECV were independently correlated with NT-proBNP \( (r^2 = 0.212, \ P < 0.001 \) and \( r^2 = 0.307, \ P < 0.001 \), respectively; Figure 5A and B) and hs-TnT \( (r^2 = 0.203, \ P < 0.001 \) and \( r^2 = 0.132, \ P < 0.001 \), respectively; Figure 5C and D).

Aortic stenosis valve severity did not associate with CVF, endocardial thickness, LGE, ECV, NT-proBNP levels, or the degree of LV remodelling. Of LGE, ECV, and CVF, only ECV correlated weakly with the patient functional limitation (6MWT; \( r^2 = -0.042, \ P = 0.040 \)), but this association was lost when adjusting for confounding factors.

To further evaluate the potential confounding effect of CAD, we performed a sensitivity analysis by excluding patients with CAD and we obtained the same results as with the adjusted multivariate linear regression analysis.

Clinical and structural impact of late gadolinium enhancement and extracellular volume fraction stratification

To compare LGE and ECV with clinical and structural parameters, we dichotomized the variables (above and below median: 10.5 g for LGE, 28.4% for ECV) with results shown in Table 2.

Patients with high vs. low LGE had more advanced LV remodelling with higher LVESVi \( (P = 0.041) \), LVEDVi \( (P = 0.045) \), LV mass index \( (P = 0.035) \), left atrial area index (LAAi) \( (P = 0.006) \), lower LVEF \( (P = 0.032) \), more mitral regurgitation \( (P = 0.012) \), higher prevalence of hypertension \( (P = 0.006) \), and CAD \( (P = 0.015) \). In accordance with
Figure 4 Association of late gadolinium enhancement with collagen volume fraction. Late gadolinium enhancement (LGE) quantified in grams by a three standard deviation method correlated strongest with collagen volume fraction (CVF) in endocardial containing samples (linear fit $y = 0.814x + 3.109$).

the association analysis, these patients presented higher CVF values ($P < 0.001$), NT-proBNP ($P < 0.001$), and hs-TnT ($P = 0.001$) levels.

Patients with high vs. low ECV also had greater LV remodelling with increased LVEDVi ($P = 0.012$), LVESVi ($P = 0.002$), and lower LVEF ($P = 0.0031$). Although the LAAi was not significantly different ($P = 0.08$), diastolic function was worse ($E/A, P = 0.022$ and $E'/e$ ratio, $P = 0.018$). Moreover, they also had an impaired 6MWT and a higher New York Heart Association functional class. In accordance with the association analysis, these patients presented higher NT-proBNP ($P < 0.001$) and hs-TnT ($P = 0.018$) levels.

Combining LGE and ECV added value (Table 3). With increasing abnormality in these parameters, cavity dimensions (LVEDVi, LVESVi, and LAAi) increased, LVEF decreased, NT-proBNP and hs-TnT levels increased, CVF increases, and patient functional capacity (6MWT) decreased ($P = 0.010$). Interestingly, these changes were maintained when we adjusted the analysis by the presence of CAD.

Discussion

In this, the largest prospective AS biopsy and multimodality imaging/bio-marker study to date, the main findings are: (i) Histological assessment of the myocardium in severe AS revealed complex morphology and topography of fibrosis with three main patterns: thickened endocardium with a massive fibrotic layer; a fibrosis gradient from the subendocardial microscars. The ECV was only mildly elevated with broadly proportional increase in the cellular and extracellular components of the myocardium (as observed by Schwarz et al. in 1978), and, unlike other papers, did not correlate with CVF. The different patterns of collagen in severe AS may have different pathogenic mechanisms and possible consequences. Most collagen deposits exist as a thickened endocardial layer and subendocardial scattered microfoci and trabecular fibrosis. Mid-myocardial fibrosis appears as a diffuse network around cardiomyocytes and bundles. The fibrosis gradient may be related to low-endocardial perfusion, thus reflecting a reparative response (i.e. replacement fibrosis) to ischaemia and subsequent cell loss. This is supported by previous findings showing that reduced capillary density, in absolute terms as well as in relation to the number of cardiomyocytes, accompanies MF in patients with severe AS. On the other hand, the diffuse MF located around cardiomyocytes may be reactive to pressure overload-induced mechanical stimulation of local fibroblasts and to paracrine factors produced by mechanically stressed (strain) cardiomyocytes that, in turn, stimulate fibroblasts (i.e. reactive fibrosis).

Cardiovascular magnetic resonance findings

Cardiovascular magnetic resonance tissue characterization has developed over two decades, initially with the LGE technique for focal fibrosis, later with the ECV technique for diffuse fibrosis. Combined biopsy and CMR study are rare and limited by small sample size. Instead, myocardial tissue characterization in AS has been described by presence or absence, and pattern of LGE (subendocardial infarct-pattern vs. mid-wall non-infarct LGE). These histological findings of thickened endocardium and a gradient of myocardial fibrosis from endo- to epicardium suggest that these descriptive LGE pattern need to be revisited, possibly by utilizing the latest motion-correction or dark blood techniques.

Here, LGE correlated with CVF (although the biopsy was obtained from the basal anteroseptum which was not infarcted in any patient), especially on endocardial biopsies ($r^2 = 0.5$), which capture more of the subendocardial microscars. The ECV was only mildly elevated with broadly proportional increase in the cellular and extracellular components of the myocardium (as observed by Schwarz et al. in 1978), and, unlike other papers, did not correlate with CVF.
Figure 5  Associations of imaging and blood biomarkers. Late gadolinium enhancement (LGE) and extracellular volume fraction (ECV) correlated with NT-proBNP (A and B) and with hs-TnT (C and D) (linear fit: A $y = 0.119x - 1.498$; B $y = 0.037x + 0.103$; C $y = 0.025x + 1.567$; D $y = 0.110x + 1.008$).

Take home figure  This figure summarizes the main findings of the study.
However, ECV did capture functionally important consequences, given that patients with high ECV showed worse NT-ProBNP, 6MWT, and NYHA functional class. There are a number of possible reasons for this discordance with other studies including the underestimation of subendocardial microscar and fibrosis gradient due to avoidance of the endo- and epicardium (for ECV we eroded 10% from the edge to avoid blood pool contamination); recruitment of a less severe (more representative) phenotypes with less extensive scarring (we recruited 50% of all AVR in our institution); reduced capillary density (lower ECV) or compensatory vasodilatation (higher ECV) may confound ECV measurements, which captures all extracellular volume including the intravascular plasma. On the other hand, diffuse scarring (we recruited 50% of all AVR in our institution); reduced capillary density (lower ECV) or compensatory vasodilatation (higher ECV) may confound ECV measurements, which captures all extracellular volume including the intravascular plasma.26,27

### Table 2: Patients stratified according to late gadolinium enhancement or extracellular volume median value

|                  | LGE |          |          | P-value |          |          |          | P-value |
|------------------|-----|----------|----------|---------|----------|----------|----------|---------|
|                  | <10.5 g (n = 65) | >10.5 g (n = 66) | P-value | <28.4% (n = 58) | >=28.4% (n = 58) | P-value |
| Age (years)      | 68.8 ± 10.4 | 71.7 ± 8.8 | 0.089 | 70.3 ± 10.0 | 70.1 ± 9.7 | 0.910 |
| Gender (male/female) | 28/37 | 45/21 | 0.004 | 34/24 | 31/27 | 0.575 |
| BMI (kg/m²)      | 28.7 ± 5.5 | 28.2 ± 4.7 | 0.567 | 27.8 ± 5.0 | 29.3 ± 5.0 | 0.097 |
| Comorbidities, n (%) |       |         |         |         |         |         |         |
| HTN              | 44 (68%) | 56 (89%) | 0.006 | 43 (75%) | 49 (84%) | 0.225 |
| AF               | 6 (9%)  | 13 (20%) | 0.089 | 7 (12%)  | 11 (19%) | 0.305 |
| CAD              | 14 (22%)| 29 (45%) | 0.015 | 19 (33%) | 21 (36%) | 0.746 |
| Symptom, n (%)   |       |         |         |         |         |         |
| Syncope          | 6 (9%)  | 4 (6%)  | 0.600 | 6 (10%)  | 4 (7%)  | 0.489 |
| NYHA             | 0.721  |          |        |          |         |         |
| I                | 8 (12%) | 9 (14%) | 0.035 | 8 (14%)  | 5 (9%)  |         |
| II               | 33 (51%)| 28 (42%)| 0.418 | 34 (59%) | 24 (41%)|         |
| III              | 17 (26%)| 23 (35%)| 0.031 | 13 (22%) | 21 (36%)|         |
| IV               | 2 (3%)  | 2 (3%)  |        | 0 (0%)   | 4 (7%)  |         |
| Valve type, bi/tri (n) | 20/45 | 18/48 | 0.659 | 16/42 | 17/41 | 0.837 |
| AVAi (cm²/m²)    | 0.41 ± 0.14 | 0.40 ± 0.13 | 0.605 | 0.42 ± 0.15 | 0.39 ± 0.11 | 0.384 |
| Mean gradient (mmHg) | 44.8 ± 11.9 | 47.8 ± 14.7 | 0.216 | 47.1 ± 14.0 | 44.8 ± 13.0 | 0.368 |
| Mitral regurgitation (%) | 7.4 ± 11.2 | 15.2 ± 14.3 | 0.012 | 9.1 ± 12.7 | 11.5 ± 12.9 | 0.428 |
| EDVi (mL/m²)     | 63.2 ± 20.9 | 70.2 ± 21.9 | 0.045 | 61.0 ± 19.1 | 71.4 ± 24.7 | 0.012 |
| ESVi (mL/m²)     | 19.0 ± 15.6 | 25.6 ± 20.7 | 0.041 | 16.9 ± 12.0 | 27.7 ± 23.3 | 0.002 |
| LVMI (g/m²)      | 83.9 ± 27.5 | 90.7 ± 20.7 | 0.035 | 83.7 ± 24.4 | 91.8 ± 25.6 | 0.085 |
| LVEF (%)         | 72.4 ± 13.2 | 67.1 ± 15.4 | 0.032 | 73.9 ± 11.4 | 65.6 ± 17.0 | 0.003 |
| MAPSE (mm)       | 10.7 ± 3.5 | 9.8 ± 3.6 | 0.123 | 11.0 ± 3.2 | 9.53 ± 3.7 | 0.031 |
| LAAi (cm²/m²)    | 12.7 ± 3.3 | 14.5 ± 4.2 | 0.006 | 12.8 ± 3.3 | 14.4 ± 4.6 | 0.081 |
| E/A              | 0.91 ± 0.42 | 1.08 ± 0.58 | 0.084 | 0.87 ± 0.38 | 1.10 ± 0.59 | 0.022 |
| DT (ms)          | 245 ± 69  | 227 ± 82  | 0.206 | 236 ± 72  | 237 ± 81  | 0.940 |
| E/e'             | 13.57 ± 6.28 | 13.94 ± 6.11 | 0.771 | 12.46 ± 6.27 | 14.94 ± 5.96 | 0.018 |
| 6MWT (m)         | 468 ± 190 | 412 ± 187 | 0.143 | 488 ± 145 | 393 ± 210 | 0.006 |
| ECV (%)          | 27.5 ± 2.6 | 29.5 ± 2.8 | <0.001 | 26.0 ± 1.7 | 30.7 ± 1.8 | <0.001 |
| LGE (g)          | 5.84 ± 2.5 | 22.8 ± 9.9 | <0.001 | 11.5 ± 9.1 | 15.4 ± 12.2 | 0.090 |
| CVF (%)          | 7.3 ± 4.7 | 15.7 ± 9.8 | <0.001 | 10.4 ± 7.5 | 10.9 ± 8.5 | 0.707 |
| NT-proBNP (pg/mL) | 96 ± 139 | 277 ± 341 | <0.001 | 99 ± 154 | 262 ± 335 | <0.001 |
| hs-TnT (ng/L)    | 15 ± 10  | 21 ± 20  | 0.001 | 15 ± 13  | 21 ± 18  | 0.018 |

Boldface values indicate statistically significant \( P \)-values.

Values are given as mean ± SD or n (and percentage).

LGE, late gadolinium enhancement; ECV, extracellular volume; BMI, body mass index; HTN, hypertension; AF, atrial fibrillation; CAD, coronary arterial disease; EDVi, end-diastolic volume index; ESVi, end-systolic volume index; LVMI, left ventricular mass index; LVEF, left ventricular ejection fraction; MAPSE, mitral annulus plane systolic excursion; LAAi, left atrial area index; E, peak early velocity of the transmitral flow; A, peak late velocity of the transmitral flow; DT, deceleration time; E', peak early diastolic velocity of the mitral annulus displacement; 6MWT, 6-minute-walk test; bi, bicuspid; tr, tricuspid; AVAi, aortic valve area index; CVF, collagen volume fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; hs-TnT, high sensitivity troponin T; SD, standard deviation; NYHA, New York Heart Association.
reactive interstitial fibrosis is intimately linked to its local environment and depends on cardiomyocyte function, strain and its interactions with fibroblasts. Extracellular volume fraction may therefore be more closely linked to the cardiomyocyte stress, and accordingly could be considered more a measure of cardiomyocyte-interstitial relationship than the current mainstream concept of ECV being a pure interstitial marker.

**Clinical impact**

Myocardial fibrosis in severe AS has a characteristic pattern and distribution. When measuring MF by biopsy or CMR, location, sampling and technical aspects of analysis matter. Invasive biopsy is limited by size and sampling error, whereas LGE and ECV capture different regions of myocardium and provide complementary information. Both

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### Table 3  Patients stratified according to extracellular volume and late gadolinium enhancement combined

|                      | ECV-/LGE- (n = 37) | ECV-/LGE+ & ECV+/LGE- (n = 46) | ECV+/LGE+ (n = 32) | P-value |
|----------------------|--------------------|---------------------------------|--------------------|---------|
| **Age (years)**      | 68.6 ± 11.0        | 71.4 ± 9.0                      | 70.4 ± 9.8         | 0.421   |
| **Gender (male/female)** | 19/18             | 24/22                           | 22/10              | 0.259   |
| **BMI (kg/m²)**      | 28.3 ± 5.3         | 28.4 ± 5.4                      | 29.0 ± 4.5         | 0.594   |
| **Comorbidities, n (%)** |                  |                                 |                    |         |
| HTN                  | 24 (65%)           | 38 (83%)                        | 29 (91%)           | **0.041** |
| AF                   | 3 (8%)             | 7 (15%)                         | 8 (25%)            | 0.243   |
| CAD                  | 7 (19%)            | 18 (39%)                        | 14 (44%)           | 0.072   |
| **Symptom, n (%)**   |                    |                                 |                    |         |
| Syncope              | 5 (14%)            | 2 (4%)                          | 3 (9.7%)           | 0.295   |
| NYHA                 |                    |                                 |                    | 0.125   |
| I                    | 5 (14%)            | 6 (13%)                         | 2 (6%)             |         |
| II                   | 24 (65%)           | 17 (37%)                        | 16 (50%)           |         |
| III                  | 16 (43%)           | 18 (39%)                        | 10 (31%)           |         |
| IV                   | 0 (0%)             | 2 (4%)                          | 2 (6%)             |         |
| **Valve type, bi/tri (n)** | 12/25             | 12/34                           | 9/23               | 0.814   |
| **AVAi (cm²/m²)**    | 0.42 ± 0.16        | 0.40 ± 0.12                     | 0.39 ± 0.13        | 0.329   |
| **Mean gradient (mmHg)** | 45.2 ± 14.1      | 46.7 ± 11.2                     | 45.8 ± 16.6        | 0.851   |
| **Mitral regurgitation (%)** | 6.8 ± 12.3     | 10.3 ± 11.4                     | 15.1 ± 15.2        | **0.042** |
| **EDVi (mL/m²)**     | 59.5 ± 20.8        | 66.8 ± 18.7                     | 73.9 ± 27.2        | **0.008** |
| **ESVi (mL/m²)**     | 15.5 ± 11.3        | 22.5 ± 17.1                     | 30.5 ± 25.9        | **0.001** |
| **LVMi (g/m²)**      | 81.8 ± 26.5        | 87.3 ± 26.1                     | 95.7 ± 20.9        | **0.023** |
| **LVEF (%)**         | 75.4 ± 9.4         | 69.0 ± 15.0                     | 63.6 ± 17.4        | **0.001** |
| **MAPSE (mm)**       | 11.3 ± 3.2         | 10.1 ± 3.5                      | 9.2 ± 3.8          | **0.014** |
| **LAAi (cm²/m²)**    | 12.4 ± 3.3         | 13.3 ± 3.1                      | 15.6 ± 5.2         | **0.001** |
| **E/A**              | 0.84 ± 0.26        | 0.97 ± 0.54                     | 1.24 ± 0.63        | **0.004** |
| **DT (ms)**          | 240 ± 68           | 243 ± 76                        | 220 ± 88           | 0.364   |
| **E/e’**             | 13.09 ± 6.83       | 13.04 ± 5.47                    | 15.60 ± 6.51       | 0.192   |
| **6MWT (m)**         | 512 ± 136          | 420 ± 207                       | 391 ± 188          | **0.010** |
| **ECV (%)**          | 25.6 ± 1.6         | 28.7 ± 2.1                      | 31.2 ± 1.9         | **<0.001** |
| **LGE (grams)**      | 6.01 ± 2.48        | 12.64 ± 9.67                    | 23.16 ± 11.22      | **<0.001** |
| **CVF (%)**          | 7.84 ± 5.01        | 10.26 ± 8.03                    | 14.45 ± 9.45       | **0.001** |
| **NT-proBNP (pg/mL)** | 60 ± 90           | 160 ± 189                       | 342 ± 394          | **<0.001** |
| **hs-TnT (ng/L)**    | 13.5 ± 14.5        | 17.5 ± 12.2                     | 23.6 ± 20.4        | **0.012** |

Boldface values indicate statistically significant P-values. Values are given as mean ± SD or n (and percentage).

LGE, late gadolinium enhancement; ECV, extracellular volume; BMI, body mass index; HTN, hypertension; AF, atrial fibrillation; CAD, coronary arterial disease; EDVi, end-diastolic volume index; ESVi, end-systolic volume index; LVMi, left ventricular mass index; LVEF, left ventricular ejection fraction; MAPSE, mitral annular plane systolic excursion; LAAi, left atrial area index; E, peak early velocity of the transmitral flow; A, peak late velocity of the transmitral flow; DT, deceleration time; E/e’, peak early diastolic velocity of the mitral annulus displacement; 6MWT, 6-minute-walk test; bi, bicuspid; tri, tricuspid; AVAi, aortic valve area index; CVF, collagen volume fraction; NT-proBNP, N-terminal of pro-brain natriuretic peptide; hs-TnT, high sensitivity troponin T; NYHA, New York Heart Association.
ECV and LGE track cardiomyocyte stress (NT-proBNP) and injury (hs-TnT). Late gadolinium enhancement is known to track troponin concentrations in AS which has been associated with advanced hypertrophy, replacement fibrosis and outcome.28 Data on BNP vs. ECV in AS are lacking. Blood biomarkers reflect ‘whole heart’ cardiomyocyte stress and injury, but need to be interpreted in conjunction with structural and functional parameters from non-invasive imaging, as they can be elevated due to other causes. The imaging biomarkers LGE and ECV offer global but also regional insights—symmetrical remodelling is common in AS and is associated with increased myocardial injury, left ventricular decompensation, and adverse events.29 The combination of LGE and ECV—a multi-parametric approach—better identified worse adverse LV remodelling, altered biochemical and histological parameters, and functional capacity than each parameter alone. Timing of AVR is one of the challenges in AS, in particular in asymptomatic patients. Recent focus has turned towards the complex interplay between the degree of the valve stenosis, haemodynamic load, and myocardial response. The combination of LGE and ECV may prove to help in a better understanding of this interplay.

**Strengths and limitations**

This is the largest combined histology-multimodality imaging study in AS, with even sub-groups larger than previous (n ~20) histological and combined studies.9–11,18–21 The analysis of the imaging and histological data was performed completely blinded by independent groups. To make this study as applicable as possible, we recruited all-comers (50% of all AVR for AS in our institution) rather than the severe end of the spectrum and thereby included patient with CAD, hypertension and diabetes. The effect of CAD was adjusted for as pertainers (50% of all AVR for AS in our institution) rather than the severe end of the spectrum and thereby included patient with CAD, hypertension and diabetes. The effect of CAD was adjusted for as well as other factors (i.e. cardiomyocytes and microvessels) also sub-group might be relevant to better characterize MF in severe AS patients. Importantly, the combination LGE and ECV allows a better phenotyping of AS patients according to their myocardial response to AS in terms of MF and morphological and functional cardiac alterations.

**Conclusion**

Myocardial fibrosis in severe AS is complex with three main alterations: endocardial thickening, subendocardial microscars, and diffuse interstitial fibrosis. Neither histological collagen volume fraction nor the CMR parameters ECV and LGE capture this fibrosis in its totality. This study supports that the combination of invasive and non-invasive techniques at scale is relevant to better characterize MF in severe AS patients. Importantly, the combination LGE and ECV allows a better phenotyping of AS patients according to their myocardial response to AS in terms of MF and morphological and functional cardiac alterations.

**Supplementary material**

Supplementary material is available at European Heart Journal online.

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**Conflict of interest**

None declared.

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