

**Regional Distribution of Extracellular Volume quantified by Cardiac CT in
Aortic Stenosis: Insights into Disease Mechanisms and Impact on Outcomes**

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ABSTRACT

Background: Extracellular volume fraction (ECV) is a marker for myocardial fibrosis and infiltration, can be quantified using cardiac CT (ECV_{CT}), and has prognostic utility in several diseases. This study aims to map out regional differences in ECV_{CT} to obtain greater insights into the pathophysiological mechanisms of ECV expansion and its clinical implications.

Methods: Three prospective cohorts were included: Patients with aortic stenosis (AS) and coexisting AS and transthyretin cardiac amyloidosis (AS-ATTR) were referred for a transcatheter aortic valve replacement and had ECG-gated CT angiography and Technetium-99m-labelled 3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy to differentiate between the two cohorts. Controls had CT angiography and cardiac magnetic resonance demonstrating no significant coronary artery disease or infarction. Global and regional ECV_{CT} was analyzed and its association with mortality assessed for patients with AS.

Results: 199 patients: controls (n=65; 66% male), AS (n=115) and AS-ATTR (n=19) had a global ECV_{CT} of 26.1 (25.0-27.8) vs 29.1 (27.5-31.1) vs 37.4 (32.5-46.6)%, respectively; $p < 0.001$. Across cohorts, ECV_{CT} was higher at the base (vs apex), the inferoseptum (vs anterolateral wall) and subendocardium (vs subepicardium); $p < 0.05$ for all. Among AS patients, epicardial ECV_{CT}, rather than any other regional value or global ECV_{CT}, was the strongest predictor of mortality at a median of 3.9 (max 6.3) years (adjusted HR: 1.21, 95% CI: 1.08-1.36; $p = 0.002$).

Conclusions: Regional differences in ECV_{CT} suggest a predilection for fibrosis and amyloid infiltration at the base, subendocardium, inferior wall and septum more than anterior and

lateral myocardium. ECV_{CT} can predict long-term mortality with the subepicardium demonstrating the strongest discriminatory power.

Keywords: aortic stenosis, extracellular volume, computed tomography, cardiac amyloidosis, myocardial fibrosis

Clinical Perspective

ECV_{CT} has demonstrated prognostic utility in several diseases. However, it can also provide important insights into disease mechanisms. Using a large prospectively recruited population, this study harnessed the high spatial resolution of ECV_{CT} to provide regional insights into disease pathophysiology and their consequent clinical implications. We identified an increase in global ECV_{CT} from controls vs AS vs AS-ATTR. We also demonstrated higher ECV_{CT} values in certain regions: base (vs apex), the inferoseptum (vs anterolateral wall) and subendocardium (vs subepicardium) across all three cohorts. This signifies a predilection for both fibrosis and amyloid in certain parts of the myocardium with three possible mechanistic explanations. Firstly, transmural homogeneity in inferoseptal ECV_{CT} suggests the pathological processes affect the right ventricular subendocardium (which is effectively the left ventricular subepicardium). Secondly, higher ECV_{CT} in the subendocardium compared to the subepicardium of the anterolateral wall can be explained by the high wall stress in this region which in patients (AS and AS-ATTR) is also the most susceptible to ischemia due to cardiac remodeling and transmural perfusion gradient. Thirdly, the higher ECV_{CT} in the base compared to the apex suggests that differential pathological involvement may explain the echocardiography-derived apical sparing pattern often seen with AS and ATTR. These regional differences have important prognostic implications. In patients with AS, epicardial ECV_{CT} provides the best discrimination for mortality compared to other regional values. This may suggest that patients with more epicardial fibrotic involvement represent advanced myocardial remodeling and consequently a higher risk subpopulation.

Non-standard abbreviations and acronyms

AS- aortic stenosis

AS-ATTR- coexisting aortic stenosis and transthyretin cardiac amyloidosis

DPD- ^{99m}Tc -3,3-diphosphono-1,2-propanodicarboxylic acid based scintigraphy

ECV_{CT}- extracellular volume quantified by computed tomography

HU- Hounsfield units

TAPSE- tricuspid annular planar systolic excursion

TAVR- transcatheter aortic valve replacement

Introduction

The extracellular space is affected by several cardiac diseases that alter its composition and consequently affect cardiac function and patient outcomes. Two key pathologies- fibrosis and amyloid influence this space. Myocardial fibrosis is the common final pathological pathway in many cardiovascular diseases and is the result of an imbalance between synthesis, deposition and degradation of collagen fibers. There are two types- focal fibrosis that represents scar and is commonly associated with myocardial infarction, and diffuse fibrosis associated with diseases such as aortic stenosis (AS) (1). Cardiac amyloidosis is the result of deposition of insoluble amyloid fibrils within the extracellular space. Both fibrosis and amyloid infiltration increase extracellular volume (ECV) which can be quantified using cardiac magnetic resonance imaging (CMR). ECV has been shown to be an important predictor of all-cause mortality and functional status in aortic stenosis and cardiac amyloidosis (2–4).

Cardiac computed tomography (CT) can quantify ECV (ECV_{CT}) and is increasingly demonstrating clinical utility both in diagnostics for screening of cardiac amyloidosis and prognosis in patients with aortic stenosis (5,6). CT has a high spatial resolution and allows the quantification of ECV according to anatomical territory (according to the American Heart Association [AHA] 17 segment model) and transmural location (epicardial, mid-myocardium and endocardial).

We have previously demonstrated among patients with AS, that fibrosis measured using ECV_{CT} can predict all-cause mortality along with right ventricular function (measured using tricuspid annular planar systolic excursion-TAPSE) in the mid-term (6). Comparing differences in regional ECV_{CT} within and between various diseases can provide mechanistic insights and refine risk stratification by assessing the impact of these differences on outcomes. The aim of this study was to exploit the superior spatial resolution of ECV_{CT} to

describe regional differences in ECV expansion in three populations representing normal myocardium, diffuse fibrosis and amyloid infiltration and evaluate which regional parameter has the highest prognostic implications.

Methods

Study population

Three patient cohorts were compared in this study: suspected coronary artery disease (controls), severe aortic stenosis and severe aortic stenosis and transthyretin cardiac amyloidosis (AS-ATTR). The control cohort comprised of consecutive, prospectively recruited patients who had chest pain and were referred for a computed tomography coronary angiogram (CTCA), as they were deemed to have a low pre-test probability for coronary artery disease. These patients also had a perfusion CMR within a fortnight of the CTCA, as part of an ongoing research protocol. Patients included in the present study as controls were those who did not have any evidence of late gadolinium enhancement on CMR, confirming an absence of prior myocardial infarction and did not have obstructive coronary artery disease on CTCA.

The AS and AS-ATTR cohorts were obtained from a prospectively recruited study- ATTRact-AS (*a study investigating the role of occult cardiac amyloid in the elderly with aortic stenosis*, NCT03029026). Details of recruitment methods and study protocols can be found elsewhere (7). In brief- patients aged 75 and over, with severe AS, referred for transcatheter aortic valve replacement (TAVR) at our center and undergoing cardiac CT as part of their clinical work-up were eligible for inclusion in this sub-study. All patients in the ATTRact-AS study underwent ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) scintigraphy as part of the study protocol and those patients with a positive result (Perugini grade 1,2 or 3) were part of the AS-ATTR cohort (8). Patients with negative DPD

scintigraphy were part of the AS cohort. Focal elevations in ECV_{CT} that corresponded to a coronary artery territory were deemed as potential infarcts. These focal ECV_{CT} elevations would skew regional ECV analysis, therefore patients with suspected infarcts were removed from this study.

Data acquisition

Demographic, clinical and echocardiographic data was obtained prospectively at baseline. All-cause mortality was obtained by searching a national database, the NHS spine, which is updated in real-time.

This sub-study complied with the Declaration of Helsinki; relevant local ethics and site approvals were obtained, and all patients provided written informed consent. The data for this study is not available for distribution due to confidentiality reasons.

Computed Tomography

Our CT protocol has been described in detail elsewhere (5). All CT scans were performed on a Somatom FORCE scanner (Siemens Healthineers, Erlangen, Germany). The acquisitions for ECV_{CT} included a pre-contrast baseline and a pseudo-equilibrium 3-minutes post-contrast scan, both prospectively triggered at 250ms after the R wave. We used the axial shuttle mode acquisition protocol for both baseline and pseudo-equilibrium scans, with a fixed tube voltage of 80kV and tube current-time product of 370mAs. Four volumes including the left ventricle were acquired every other heartbeat, during a single breath hold. The reconstruction field of view was set with the same x,y,z coordinates for both datasets. An additional dataset was reconstructed from the clinical CTCA, at 250ms of the R-R interval, with a reconstruction field of view matching that of the axial shuttle mode datasets.

Extracellular volume analysis

Our approach to analysis has been previously described elsewhere (5). Non-rigid registration software (Hepacare, Siemens Healthineers, Erlangen, Germany) allowed averaging of the four volumes and aligning of the baseline and pseudo-equilibrium axial shuttle mode datasets, to improve image quality and reduce noise. The averaged baseline and post-contrast images were registered with the CTCA image. Subsequently, the averaged baseline image was subtracted from the averaged post-contrast image, providing a partition coefficient. A region of interest was placed in the left ventricular blood pool on the CTCA image, and the hematocrit inputted, generating a myocardial ECV map via the formula: $ECV_{CT} = (1 - \text{hematocrit}) \times (\Delta HU_{\text{myo}} / \Delta HU_{\text{blood}})$, where ΔHU is the change in Hounsfield unit attenuation pre- and post-contrast (i.e. $HU_{\text{post-contrast}} - HU_{\text{pre-contrast}}$) for the myocardium (ΔHU_{myo}) and the blood (ΔHU_{blood}). A prototype software (Cardiac Function, Siemens Healthineers, Erlangen, Germany), was used to superimpose the ECV volume data onto the left ventricle and the results were displayed and numerically exported as American Heart Association 17-segment polar maps (figure 1). Segment 17, the true apex was discounted from ECV_{CT} analysis as it tends to be thin and easily contaminated by the blood pool or extra-cardiac structures. Segments with significant beam hardening artefact from adjacent pacing wires (n=4 patients) were excluded.

Study definitions and endpoints

Global ECV_{CT} refers to the average ECV for a patient based on all regions (endocardial and epicardial). Regional ECV_{CT} is described either along the radial axis: endocardial and epicardial or along the longitudinal axis: base, mid and apex or according to sections of a 17 segment AHA model (figure 1). Along the radial axis, ECV_{CT} is calculated according to the percentage of the myocardium in the radial profile; endocardial (10-50% of the inner myocardium), and subepicardium (50-90% of the outer myocardium) (figure 1). The innermost

and outermost 10% of the myocardium were not used for calculating ECV_{CT} due to possible contamination from the blood pool (spillover) and pericardium/extra-cardiac tissue, respectively. Transmural ECV_{CT} difference refers to the difference between endocardial and epicardial ECV_{CT} . Base to apex ECV_{CT} difference refers to the difference between basal and apical ECV_{CT} . Regional differences were also calculated and presented here as the difference in ECV_{CT} for a particular region compared to the global ECV_{CT} for that patient. In order to deduce clinical implications of regional differences in ECV_{CT} , prediction models were created to evaluate the association of regional ECV on all-cause mortality for the AS cohort that underwent a TAVR. Patients who were managed medically were excluded from this analysis as their outcomes are known to be worse. The control cohort was not included in the mortality analysis due to significant lower age and short duration of follow-up. Focus of the outcome analysis was the effect of fibrosis on outcome, therefore patients with dual pathology AS-ATTR were excluded (this is unique to other ECV_{CT} studies (9)); this cohort per se was too small to make any inferences regarding mortality.

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software (Version 19.1.3, MedCalc Software Ltd., Ostend Belgium) and R version 4.0.2 (R Foundation for Statistical Computing). Where appropriate, results are described either as mean $\pm$ standard deviation or median (interquartile range). The Mann-Whitney U test was used to compare two independent continuous variables, Kruskal-Wallis test for more than two independent variables with inter-group comparison and the Friedman's test for comparison of more than two dependent variables. Either Chi-Squared or Fisher's Exact testing was used for categorical data as appropriate. Once regional differences in ECV_{CT} were identified, their potential prognostic relevance was determined for patients in the AS cohort. The overall impact of ECV_{CT} on

survival was assessed by Kaplan-Meier analysis of patients stratified according to the global median population ECV_{CT} value. Significance was tested using the Peto & Peto modification of the Gehan-Wilcoxon test (10). A detailed analysis of the prognostic impact of regional ECV_{CT} parameters and competing demographic and functional parameters was done by univariable and multivariable Cox regression. The proportionality assumption of the Cox regression models was verified by performing the individual and global Schoenfeld test for all variables and combinations used. A two-sided *p*-value <0.05 was considered statistically significant.

Results

Baseline characteristics

199 patients were included in this study: 65 controls, 115 with AS and 19 with AS-ATTR; age 59 (53- 67), 85 (81- 88), 88 (85- 90) respectively, *p*<0.001; male sex 66%, 46%, 63% respectively, *p*=0.025. Among patients with AS-ATTR, 6 had Perugini grade 1, 12 had Perugini grade 2 and 1 had Perugini grade 3. Other demographic, comorbidities and basic echocardiographic data are presented in table 1. Supplementary figure S1 demonstrates how this study population was derived.

ECV_{CT} between cohorts

The median global ECV_{CT} for controls, AS and AS-ATTR was 26.1 (25.0-27.8) vs 29.1 (27.5-31.1) vs 37.4 (32.5-46.6)% respectively; *p*<0.001. Global transmural ECV_{CT} absolute difference was 2.0 (1.1-3.0) vs 4.3 (3.0-5.7) vs 4.4 (3.5-6.1) respectively; *p*<0.001. ECV_{CT} at base, mid, and apex was lower in controls compared to the respective regions in the AS and AS-ATTR cohorts (*p*<0.05 for both). Whereas there was no statistical difference between the AS and AS-ATTR cohorts (table 2).

ECV_{CT} within cohorts

ECV_{CT} was higher in the base among all cohorts, reduced in the mid myocardium and was lowest at the apex ($p=0.001$) (table 2 and figure 2). The highest ECV_{CT} was documented in the inferior wall and septum, whilst the lowest ECV_{CT} was documented in the lateral and anterior walls in all three cohorts (table 3 and figure 3). The anterior and lateral wall demonstrated the largest transmural ECV_{CT} difference in all three cohorts with the subendocardium demonstrating the highest ECV_{CT} and subepicardium the lowest. The inferior wall and septum demonstrated the least transmural difference (i.e. subendocardium was similar to subepicardium) (table 3 and figure 4 and supplementary figure S2).

Impact of regional ECV_{CT} on mortality

Among the AS cohort, there were 43 deaths after TAVR ($n=95$) at a median follow-up of 3.9 years (maximum follow-up 6.3 years). ECV_{CT} in all regions predicted mortality, however, epicardial ECV_{CT} proved to be the best discriminator- hazard ratio (HR): 1.21, 95% confidence interval (CI): 1.08-1.36; $p=0.001$ (Table 4). Among other clinical variables, only TAPSE predicted mortality. After adjusting each regional ECV_{CT} value for TAPSE, epicardial ECV_{CT} remained the strongest predictor of mortality (HR: 1.21, 95% CI: 1.08-1.36; $p=0.002$) (Table 5). There was no indication of a relevant violation of the proportional hazard assumption. The lowest p-value of individual and global Schoenfeld test was 0.2; for the most relevant variables, epicardial ECV_{CT} and TAPSE, p was greater 0.5.

Among the AS cohort, patients were stratified according to median ECV_{CT} (27.1%). Patients with low ECV_{CT} had a better survival compared to those with high ECV_{CT}, weighted Log Rank $P=0.006$ (Figure 5). Of the 13 patients in the AS-ATTR cohort who underwent a TAVR, 12 died during follow-up.

Discussion

Exploiting the 256x higher spatial resolution of ECV by CT (over CMR), we were able to demonstrate that (1) ECV not only increases with afterload and infiltration, but that there are (2) regional difference in ECV with base to apex and transmural gradients both in health and disease. Location of the ECV measurement is critical for the detection of the greatest disease activity (fibrosis/infiltration), but also because (3) different locations have different strengths of association with all-cause mortality. These findings, if confirmed in larger and more diverse cohorts have important implications for future ECV guided studies and trials.

Advances in cardiac CT now provide novel insights into myocardial characterization- an area largely dominated by CMR. CT provides the added advantages of greater availability, shorter examination times and being better tolerated by claustrophobic patients. However, CT has inferior contrast resolution and currently characterization of focal scar is in its infancy compared to CMR. In our study, we were able to identify focal scar using ECV_{CT} , corresponding to specific coronary territory. Others have done the same (11,12). However, further work including validation needs to be carried out to establish focal scar assessment using CT. For patients that routinely undergo cardiac CT such as those with AS requiring CT for TAVR planning, ECV_{CT} can provide valuable additional information at low additional radiation (2 to 3mSv) and examination time (3 additional minutes)- making it a convenient adjunct. There is still a lack of consensus about the minimum technical requirements, the optimal contrast and timing protocol for performing reliable ECV_{CT} measurements. We have used a dual source scanner with high temporal resolution allowing for end systolic prospective triggering. This provides a thicker myocardium and is essentially immune to heart rate variations and ectopic beats; we did not exclude any patients and all scans were diagnostic. The

remaining workflow complexity of having to do an initial baseline scan, a delayed scan and then perform a registered subtraction of both scans may be streamlined with photon-counting CT that routinely generates delayed iodine maps and therefore eliminates this step, facilitating integration of ECV_{CT} into clinical practice (13,14).

The extracellular space represents the vascular bed, matrix proteins and fibrous tissue. In patients with AS-ATTR, ECV_{CT} is known to be higher due to a combination of both transthyretin amyloid fibrils and excessive myocardial fibrosis. Whereas in AS it represents an elevated fibrotic burden (15,16). We have demonstrated that increased ECV_{CT} can be used to screen for ATTR among patients with AS with considerably high diagnostic accuracy (5). With a prevalence of 1 in 8 patients, coexisting ATTR is important to diagnose especially because of its therapeutic implications and affects outcomes (17,18). The phenotype of AS-ATTR has many similarities to ATTR, suggesting a role for ATTR-specific medications (19). Both Tafamidis and Patisiran have demonstrated safety and efficacy among patients with ATTR (20,21). ECV_{CT} is the ideal screening tool for identifying patients with AS-ATTR, as all TAVR patients will undergo a CT during the planning phase. Further studies investigating the role of disease modifying medications such as Tafamidis and Patisiran in patients with AS-ATTR are required.

Our findings regarding regional difference in ECV_{CT} provide further insight into disease mechanisms. Transmural homogeneity in inferoseptal ECV_{CT} suggests the pathological processes affect the entire radial width of the myocardium in this region. Whereas the higher ECV_{CT} in the subendocardium compared to the subepicardium of the anterolateral wall suggests a predilection for the pathological processes to affect the subendocardium. The subendocardium is subjected to high wall stress and in patients (AS and AS-ATTR) is most

susceptible to ischemia due to cardiac remodeling and changes in coronary hemodynamics (22). Both these factors are likely to lead to higher myocyte death within the subendocardium and resulting in increased fibrosis (16,22). Amyloid deposition is thought to be driven by a mechano-enzymatic process where increased wall stress promotes deposition (23). Within the septum, the left ventricle's subepicardium represents the right ventricle's subendocardium. Patients with AS can develop right ventricular dysfunction and pulmonary hypertension (24), which may impose increased wall stresses on the right ventricular subendocardium, albeit to a lesser magnitude compared to the left ventricle. This may explain the homogeneity of ECV_{CT} in the septum.

In ATTR, an apical sparing pattern is seen with global longitudinal strain (GLS) and is a useful diagnostic and prognostic marker (25,26). Late gadolinium enhancement using cardiac magnetic resonance has shown a similar pattern (27) and so has ^{99m}Techetium-Pyrophosphate (PYP) scintigraphy. The latter demonstrated prognostic implications of apical sparing (28). The apical sparing pattern has three possible explanations; the base has more amyloid deposition, increased myocyte death and/or less diversity of myocyte and matrix orientation (23). In AS, an apex to base gradient of GLS has been described, although to a lesser extent than in ATTR (25,29). Our study demonstrates a similar apical sparing pattern in both AS and AS-ATTR patients using ECV_{CT}. Although speculative, this suggests that regional differences in fibrosis and amyloid infiltration may explain regional functional differences with GLS. However, this correlation needs to be proven by future studies.

We have previously showed that ECV_{CT} can predict mortality in the mid-term among patients with AS (6). Using ECV_{CT} to identify such patients may improve the targeting of such therapies. Our data also shows that regional differences in ECV_{CT} may improve risk

stratification. Fibrosis and amyloid infiltration may progress from the subendocardium towards the subepicardium, such that with advanced disease the subepicardium is affected, whereas mild disease has predominantly endocardial involvement. This may explain why epicardial ECV_{CT} in our study carries the strongest prognostic value over other regional ECV_{CT} values. Integrating epicardial ECV_{CT} into risk stratification of TAVR patients may better inform clinicians and patients about possible outcomes. ECV_{CT} could also play an important role in optimizing the timing of valve replacement. CMR based studies have identified worse outcomes in AS patients with late gadolinium enhancement and higher ECV (3,30,31). This has been the impetus for a multicenter, randomized controlled trial evaluating early aortic valve replacement in patients with asymptomatic severe AS, based on the presence of mid wall fibrosis (EVoLVeD, NCT03094143). In a similar way, if other studies confirm our findings, an elevated ECV_{CT} could be used in a trial to instigate aortic valve replacement in patients with moderate AS or asymptomatic severe AS.

Limitations

Although this was a single center study, we presented findings from a large population that was prospectively recruited (n=199), including a less understood cohort of patients- AS-ATTR. Despite this our sample size is limited, and validation is required in larger studies. We have not correlated ECV_{CT} to GLS in our study so our speculation regarding the apical sparing distribution of ECV_{CT} to explain similar findings from other studies should be considered hypothesis generating. Similarly, we have not provided histological evidence of right ventricular wall stress and endocardial involvement with fibrosis and amyloidosis, to support our rationale for septal ECV_{CT} homogeneity. Further studies need to investigate whether the lack of transmural ECV_{CT} in the septum is the result of right ventricular disease.

Conclusion

Regional differences in ECV_{CT} suggest a predilection for fibrosis and amyloid infiltration at the base more than the apex, subendocardium more than subepicardium and inferior wall and septum more than anterior and lateral myocardium. ECV_{CT} can predict mortality in the long-term among AS patients, with the subepicardium demonstrating the highest predictive power. The clinical applicability of ECV_{CT} is broad, from screening for coexisting ATTR, understanding disease mechanisms, refining risk stratification, and potentially optimizing aortic valve replacement.

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The remaining authors have no relevant disclosures.

Supplemental Material

Supplementary figure S1 and S2

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Tables

Table 1: Baseline characteristics are compared across the three cohorts- controls, aortic stenosis (AS) and dual pathology- AS and cardiac amyloidosis (AS-ATTR).

Parameters	Controls (n=65)	AS (n=115)	AS-ATTR (n=19)	P value
Age (years)	59 (53-67)*†	85 (81-88)	88 (85-90)	<0.001
Male sex	43 (66%)*	53 (46%)	12 (63%)	0.025
Hypertension	39 (60%)	86 (75%)	15 (79%)	0.078
Hyperlipidemia	50 (77%)*†	59 (51%)	10 (53%)	0.003
Diabetes Mellitus	25 (38%)*†	27 (23%)	3 (16%)	0.047
BMI (m/kg2)	29 (26- 32)*	26 (23-30)	27 (25-31)	0.013
Creatinine (mmol/L)	82 (70- 93)*†	99 (83-120)	117 (96-142)	<0.001
AV max (m/s)	N/A	4.1 (3.9-4.5)	4.1 (3.8-4.5)	0.447
Mean gradient (mmHg)	N/A	41 (34-48)	38 (29-47)	0.263
AVA (cm2)	N/A	0.7 (0.6-0.9)	0.7 (0.6-0.9)	0.918
LVEF (%)	N/A	58 (53-63)	55 (49-65)	0.704
TAPSE (cm)	N/A	2.0 (1.7-2.3)	1.9 (1.6-2.2)	0.524
Anteroseptal wall thickness (cm)	N/A	1.2 (1.1-1.4)	1.4 (1.3-1.6)	0.004
Inferolateral wall thickness (cm)	N/A	1.1 (0.9-1.3)	1.3 (1.1-1.4)	0.002
Left ventricular end diastolic dimension (cm)	N/A	4.4 (4.0-4.9)	4.4 (3.8-4.8)	0.941

Comparison of baseline characteristics between controls, AS and AS-ATTR was performed using Mann-Whitney U test or Kruskal-Wallis test with intergroup comparisons for non-parametric continuous variables and Chi square/Fisher's exact test for binary variables as

appropriate. Data is presented as number (percentage) for frequencies and median (interquartile range) for continuous data.

BMI- body mass index, AVA- aortic valve area, AV max- peak transaortic valve velocity, LVEF- left ventricular ejection fraction, TAPSE- tricuspid annular planar systolic excursion.

*= $p < 0.05$ for controls vs AS

†= $p < 0.05$ for controls vs AS-ATTR

Table 2: Regional ECV_{CT} values in % among patients with aortic stenosis (AS), dual pathology AS and cardiac amyloidosis (AS-ATTR) and controls.

Region	Controls (n=65)	AS (n=115)	AS-ATTR (n=19)
Base (%)	26.5 (25.3-28.0)	29.9 (28.3-32.0)	41.4 (33.1-48.1)
Mid (%)	26.2 (24.7-27.7)	28.9 (27.0-30.6)	36.3 (32.9-46.0)
Apex (%)	25.7 (24.1-27.2)	28.1 (26.3-29.9)	36.1 (31.2-44.6)
Subendocardium (%)	27.2 (26.0-28.7)	31.2 (29.3-33.9)	41.1 (35.4-47.7)
Subepicardium (%)	24.8 (24.0-26.8)	27.2 (25.2-28.9)	36.2 (29.5-45.4)

Data is presented as median (interquartile range).

Table 3: Regional differences in ECV_{CT} in % among patients with aortic stenosis (AS), dual pathology AS and cardiac amyloidosis (AS-ATTR) and controls.

ECV by Region (%)	Cohort		
	Controls (n=65)	AS (n=115)	AS-ATTR (n=19)
Anterior (%)	25.8 (24.3-27.9)*†	28.3 (26.3-30.1)*†#	37.1 (30.9-44.3)*†
Lateral (%)	25.5 (23.9-26.6)‡§	28.7 (27.0 - 31.0)‡§	37.7 (32.6-41.1)‡§
Inferior (%)	27.5 (26.0-29.5)	30.9 (29.3-33.2)	40.4 (37.1-48.9)
Septal (%)	26.8 (25.6-28.1)	29.5 (27.7-31.3)	41.2 (32.7-49.4)

Data is compared between regions within a cohort using Friedman's test. Data is presented as median (interquartile range).

*= p<0.05 for anterior vs inferior

†= p<0.05 for anterior vs septal

‡= p<0.05 for lateral vs inferior

§= p<0.05 for lateral vs septal

||= p<0.05 for inferior vs septal

#= p<0.05 for anterior vs lateral

Table 4: Univariable Cox model of all-cause mortality among the AS cohort by 3.9 years
(maximum follow-up 6.3 years).

	Hazard ratio [95% confidence interval]	P value	Change in parameter associated with a 50% increase in hazard for mortality
Age	-	0.588	-
Gender	-	0.413	-
LVEF	-	0.562	-
LVEDD		0.625	
TAPSE	0.40 [0.20,0.79]	0.008	-0.44 cm
Total ECV _{CT}	1.18 [1.05,1.31]	0.004	2.45 %
Epicardial ECV _{CT}	1.21 [1.08,1.36]	0.001	2.13 %
Endocardial ECV _{CT}	1.13 [1.02,1.24]	0.018	3.32 %
Anterior ECV _{CT}	1.16 [1.05,1.27]	0.004	2.73 %
Lateral ECV _{CT}	1.13 [1.02,1.25]	0.018	3.32 %
Inferior ECV _{CT}	1.13 [1.03,1.25]	0.011	3.32 %
Septal ECV _{CT}	1.18 [1.06,1.32]	0.002	2.45 %

LVEF- left ventricular ejection fraction, LVEDD- left ventricular end diastolic dimension,
TAPSE- tricuspid annular planar systolic excursion.

Table 5: Multivariable Cox regression for all-cause mortality among the AS cohort by 3.9 years (maximum follow-up 6.3 years).

	ECV HR [95% CI]	P value	TAPSE HR [95% CI]	P value
Total ECV _{CT}	1.13 [1.00,1.26]	0.046	0.47 [0.24,0.94]	0.034
Epicardial ECV _{CT}	1.21 [1.08,1.36]	0.002	-	0.057
Endocardial ECV _{CT}	-	0.127	0.40 [0.20,0.79]	0.008
Anterior ECV _{CT}	1.12 [1.02,1.24]	0.025	0.45 [0.23,0.88]	0.020
Lateral ECV _{CT}	-	0.130	0.40 [0.20,0.79]	0.008
Inferior ECV _{CT}	-	0.124	0.40 [0.20,0.79]	0.008
Septal ECV _{CT}	1.13 [1.01,1.27]	0.027	0.48 [0.24,0.98]	0.043

Figure legends

Figure 1. Nomenclature used in this study. A) represents a 17 segment AHA model and depicts in green the segments that account for base, mid and apex. The greyed out segment 17 was not included for ECV_{CT} analysis. B) defines the extent of the myocardium, based on wall thickness that represents the endocardium (10-50%), epicardium (50-90%) and mid-myocardium (25-75%) delineated by the checkered pattern. C) is a 17 segment AHA model that illustrates which segments make up the inferoseptum and anterolateral wall. Heart vector obtained from www.vectez.com.

Figure 2: Base to apex ECV_{CT} difference calculated as the difference between ECV_{CT} at the base and apex. All three cohorts demonstrated higher ECV_{CT} at the base compared to the apex. AS-ATTR: aortic stenosis and transthyretin cardiac amyloidosis, AS: aortic stenosis.

Figure 3: ECV_{CT} regional distribution illustrated as median and interquartile ranges and calculated as average of the difference between the specific regional ECV_{CT} (anterior, lateral, inferior and septal) and the global ECV_{CT} for that patient. AS-ATTR: aortic stenosis and transthyretin cardiac amyloidosis, AS: aortic stenosis.

Figure 4: Subendocardium to subepicardium (transmural) difference displayed as median and interquartile range for each cohort in each region. The difference is calculated as the difference between subepicardium and subendocardium. The largest transmural differences are seen in the anterior and lateral walls and smallest seen in the inferior and septum. AS-ATTR: aortic stenosis and transthyretin cardiac amyloidosis, AS: aortic stenosis.

Figure 5: Kaplan Meier analysis of AS patients with low compared to higher ECV based on the median value in our study population. The figure demonstrates higher long term mortality among patients with higher ECV_{CT} but similar outcomes up to 2 years.