Myocardial edema and prognosis in amyloidosis

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ABSTRACT

Background: Prognosis in light chain(AL) and transthyretin(ATTR) amyloidosis is influenced by cardiac involvement. ATTR has better prognosis than AL despite more amyloid infiltration, suggesting additional mechanisms of damage in AL amyloidosis.

Objective: The aim of this study was to assess the presence and prognostic significance of myocardial edema in patients with amyloidosis.

Methods: We recruited 286 patients (100 with systemic AL amyloidosis, 163 with cardiac ATTR amyloidosis, 12 with suspected cardiac ATTR amyloidosis (grade 1 on ^{99m}Tc-DPD), 11 asymptomatic individuals with amyloidogenic transthyretin (TTR) mutations) and 30 healthy volunteers. All subjects underwent CMR with T1 and T2 mapping and 16 underwent endomyocardial biopsy.

Results: Myocardial T2 was increased in amyloidosis with the degree of elevation being highest in untreated AL patients (AL untreated 56.6±5.1ms; AL treated 53.6±3.9ms; ATTR 54.2±4.1ms; each $p<0.01$ compared to controls: 48.9±2.0ms). Left ventricular (LV) mass and ECV were higher in ATTR compared to AL whilst LV ejection fraction was lower ($p<0.001$). Histological evidence of edema was present in 87.5% of biopsy samples ranging from 5% to 40% myocardial involvement. Using Cox regression models, T2 predicted death in AL amyloidosis (hazard ratio, HR,1.48, 95%CI 1.20-1.82) and remained significant after adjusting for ECV and NT-proBNP (HR 1.32, 95%CI 1.05-1.67).

Conclusions: Myocardial edema is present in cardiac amyloidosis by histology and CMR T2 mapping. T2 is higher in untreated AL amyloidosis compared to treated AL and ATTR, and is a predictor of prognosis in AL amyloidosis. This suggests mechanisms additional to amyloid infiltration contributing to mortality in amyloidosis.

KEYWORDS: Amyloidosis, CMR, T2 mapping

CONDENSED ABSTRACT: Transthyretin(ATTR) amyloidosis has a better prognosis than light chain(AL) amyloidosis despite greater amyloid infiltration. We aimed to assess the presence and significance of myocardial edema in patients with cardiac amyloidosis using CMR T2 mapping. In 286 patients, myocardial T2 was increased in amyloidosis, the degree of elevation being highest in untreated AL patients (56.6±5.1ms) compared to treated AL (53.6±3.9ms), ATTR (54.2±4.1ms) and controls (48.9±2.0ms). T2 values predicted death in AL amyloidosis after adjusting for ECV and NT-proBNP (HR 1.32, 95%CI 1.05-1.67). Myocardial edema is present and predicts outcome in AL amyloidosis, suggesting mechanisms in addition to infiltration contribute to mortality.

ABBREVIATIONS

^{99m}Tc-DPD = ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid
AL = light-chain amyloidosis
ATTR = transthyretin amyloidosis
CMR = cardiovascular magnetic resonance
ECV = extracellular volume fraction
LGE = late gadolinium enhancement
LV = left ventricular
ShMOLLI = shortened modified Look-Locker inversion recovery
NT-proBNP = N-terminal pro-B-type natriuretic peptide

TTR = transthyretin

Introduction

Systemic amyloidosis is a fatal disease characterized by progressive deposition of abnormal, insoluble protein fibrils in the extracellular space, which disrupts normal tissue architecture and function (1). The associated progressive increase in ventricular mass and stiffness (2) are thought to explain much of the pathophysiology. Nearly all cardiac amyloidosis is monoclonal immunoglobulin light-chain (AL or primary systemic) type or transthyretin (ATTR, formerly 'senile') type, with cardiac involvement being the major determinant of survival in both. Whist there are currently no proven disease modifying therapies available for ATTR amyloidosis, the prognosis of AL amyloidosis is improved with chemotherapy that suppresses aberrant light chain production from the underlying plasma cell clone in bone marrow(3). However, despite novel chemotherapy agents, 1-year mortality in established cardiac AL amyloidosis remains poor reflecting late diagnosis and need for better tolerated, less toxic and more rapidly acting therapies (4).

Cardiovascular magnetic resonance (CMR) can be used to non-invasively measure cardiac amyloid burden. CMR can visualize, with late gadolinium enhancement (LGE), and quantitate, with T1 mapping, the continuum of cardiac amyloid deposition (5). Transmurality of LGE, elevation in native T1 and extracellular volume fraction (ECV) all correlate with amyloid burden and provide incremental information on outcome (5). Amyloid infiltration in cardiac ATTR amyloidosis is usually more severe than in AL in terms of ventricular wall thickness, left ventricular (LV) mass, transmurality of LGE and ECV (6). However, severity of clinical heart failure and survival are generally worse in AL amyloidosis (7) (median survival from presentation 6 months in AL and 6 years in ATTR (2)). This discordance remains poorly

understood(7) but has been ascribed to additional toxic effects of AL amyloid(8) or faster rate of amyloid deposition in AL compared to ATTR, leading to increased myocardial damage.

T2 is a CMR biomarker that increases in myocardial edema, for example in acute myocardial infarction(9) and myocarditis(10), but also in heart failure, where it appears to track chronic inflammation(11) and cardiac Fabry disease, where T2 may be elevated in LGE areas and tracks blood troponin release(12). Whilst T2 ratio has been assessed in cardiac amyloidosis(13,14), T2 mapping has not previously been studied. Furthermore, no histological studies have demonstrated the presence of myocardial edema in cardiac amyloidosis.

The aim of this study was to assess the presence of myocardial edema using histology and T2 mapping in patients with amyloidosis and assess the prognostic significance in AL and ATTR subtypes.

Methods

Patients with amyloidosis were recruited and underwent comprehensive assessment at the National Amyloidosis Centre, Royal Free Hospital, London, United Kingdom from 2011 to 2015. Patients were systematically followed up from date of CMR until 22nd March 2017, the date of censoring. Accuracy of occurrence and date of death among deceased patients, and ongoing survival among those who were censored, was ensured on the basis of UK death certification data from the UK Office of National Statistics. A total of 286 patients were categorized into 3 groups:

AL amyloid patients: 100 patients with biopsy proven systemic AL amyloidosis (61 male, mean age 63.6±10.9 years) were recruited. Cardiac categorization was based on CMR findings (5). Categories were: a)cardiac amyloidosis with transmural LGE: features on CMR consistent with cardiac amyloidosis and transmural LGE; b)cardiac amyloidosis with

subendocardial LGE: features on CMR consistent with cardiac amyloidosis and subendocardial LGE; c)no evidence of cardiac involvement: normal wall thickness on CMR with normal serum biomarkers and no LGE. Patients within groups a) and b) were considered to have definite cardiac involvement. Patients were also classified as treated or untreated. Untreated patients were defined as those that underwent CMR scanning prior to initiation of chemotherapy to treat AL amyloidosis or associated myeloma. Treated patients were those who had completed or were currently undergoing chemotherapy.

ATTR amyloidosis patients. 163 consecutive patients (139 male, mean age 74.6±8.1 years) with cardiac ATTR amyloidosis and 12 with possible cardiac ATTR amyloidosis (8 male, age 73.3±14.4 years) were recruited. Cardiac ATTR amyloidosis was defined as the combination of heart failure symptoms with echocardiography consistent with or suggestive of cardiac amyloidosis, grade 2 or 3 cardiac uptake on the ^{99m}Tc-DPD scintigraphy in the absence of a monoclonal gammopathy or, in the presence of monoclonal gammopathy, a cardiac biopsy positive for TTR(15). Possible cardiac ATTR amyloidosis was defined by grade 1 cardiac uptake on ^{99m}Tc-DPD. All subjects underwent sequencing of exons 2, 3, and 4 of the TTR gene.

TTR gene mutation carriers. Eleven TTR gene mutations carriers were recruited (4 male, 36%; age 45.6 ± 7.5 years). These were defined as individuals with TTR gene mutation but no evidence of clinical disease, no cardiac uptake on ^{99m}Tc-DPD scintigraphy and normal echocardiography, N-terminal pro-brain natriuretic peptide (NT-proBNP) and Troponin T.

Healthy volunteers: 30 healthy volunteers (21 male, mean age 54.4±12.3 years) were recruited through advertising in the hospital, university and general practices. All had no history of cardiovascular disease, hypertension or diabetes mellitus. All had normal 12-lead ECG and normal CMR scan.

All amyloidosis patients underwent transthoracic echocardiography, 12-lead ECG and 6-minute walk test where health and patient choice permitted (e.g. not performed in the presence of severe arthritis, postural hypotension or neuropathy), and provided blood samples for NT-proBNP. The ATTR group also underwent ^{99m}Tc-DPD scintigraphy. Nineteen patients underwent clinically indicated endomyocardial biopsy (for example, if biopsies from other sites had failed to confirm the presence and type of amyloidosis).

The study was approved by the University College London/University College London Hospital Joint Committees on the Ethics of Human Research, and all participants provided written informed consent.

Exclusion Criteria: Patients with standard contraindications to CMR or glomerular filtration rate <30ml/min/1.73m^2 were excluded.

CMR protocol: All participants underwent CMR on a 1.5T scanner (Avanto, Siemens Healthcare, Erlangen, Germany). A standard volume and LGE study was performed. The gadolinium-based contrast agent used was 0.1mmol/kg gadoterate meglumine (Dotarem, Guerbet SA, Paris, France). The LGE sequence used was either standard FLASH-IR (fast low-angle single-shot inversion recovery) or true-FISP (fast imaging with steady state free precession) sequence with phase-sensitive IR or magnitude reconstruction. For native T1 mapping, basal and mid ventricular short axis, and 4-chamber long-axis were acquired using the shortened modified look-locker inversion recovery (ShMOLLI) sequence after regional shimming, as previously described(16). Post-contrast T1 mapping was performed using the same sequence and slice positions. For T2 mapping, a 4-chamber long-axis matching the T1 map was acquired using an investigational prototype (WIP 448B, Siemens Healthcare). This sequence(17) uses 3 single-shot T2-prepared steady state free precession (SSFP) readouts each separated with 3 heart beats for

T1 recovery. The echo times (TE) for the individual T2 preparations were 0, 25, and 45 milliseconds. A mono-exponential fit to a 2-parameter model, S= PDexp(-TE/T2), was used at each pixel to estimate proton density (PD) and T2.

CMR image analysis: All analysis was performed offline. LGE was graded as none, sub-endocardial or transmural as previously described(5). T1 and T2 measurements were performed by drawing a region of interest (ROI) in the basal to mid septum of the 4-chamber map. For ECV measurement, a single ROI was drawn in each of the 4 required areas: myocardial T1 estimates (basal to mid septum in 4-chamber map) and blood T1 estimates (LV cavity blood pool in 4-chamber map, avoiding the papillary muscles) before and after contrast administration. Hematocrit was taken immediately before each CMR study. ECV was calculated as: myocardial ECV = (1-haematocrit) x ($\Delta R1_{myocardium}/\Delta R1_{blood}$), where R1=1/T1 (18).

Endomyocardial Biopsies: Biopsies were performed in 19 patients. All procedures were performed percutaneously under fluoroscopic guidance and samples obtained from the right ventricle. Samples were stained with haemotoxylin and eosin (HE) and interpreted by an experienced Histopathologist (MS), blinded to the CMR results. Observations were graded for presence and extent of edema. To confirm presence of amyloid, histological analysis was performed by Congo red staining on 6μm formalin-fixed and paraffin-embedded sections, and viewed in bright-field, cross-polarized and fluorescent light. Three HE samples were unsuitable for analysis due to insufficient material so edema assessment was performed for 16 samples. Samples were also labelled with monospecific antibodies (CD68 for macrophages and CD45 for leukocytes) to assess for myocardial inflammation, which was defined as ≥14 infiltrating immune cells/mm^2 (10,19,20). Six historical myocardial biopsy samples stained with HE from

patients with confirmed acute myocarditis were also analysed for the presence of edema and inflammation.

Statistical analysis: Statistical analysis was performed using SPSS Statistics Version 24 (IBM, Somers, New York). All continuous variables were normally distributed (Sharipo-Wilk), other than NT-proBNP and C-reactive protein, which were therefore log-transformed for bivariate testing; continuous variables are presented as mean ± standard deviation (SD) with non-transformed NT-proBNP and C-reactive protein as median and interquartile range. Comparisons between groups were performed by one-way analysis of variance (ANOVA), after checking for homogeneity of variance, with post-hoc Bonferroni corrected pairwise comparisons. Linear association between T2 and other markers was assessed using Pearson's correlation coefficient for continuous variables and Spearman's correlation coefficient for ordinal variables. Cox proportional hazards regression analysis with univariable and multivariable modelling was used to assess predictors of mortality. Considering the low number of events, in order to avoid overfitting, separate multivariable models were performed, each with T2 and two other clinically relevant predictors. The log-rank test and Kaplan-Meier survival graph were used to compare groups with elevated and normal T2.

Results

Two hundred and eighty-six patients were enrolled. Baseline characteristics are shown in **Table 1**. The TTR mutations were: V122I [n = 32], T60A [n = 22], V30M [n = 10], S77Y [n = 2], and E54G, E89K, D38Y, G47V, E89L, I84S, I107F and L12P in one case each. Of the AL patients, 54% were new referrals and had not commenced chemotherapy at the time of CMR scanning ("untreated AL"). The remainder were undergoing or had completed chemotherapy ("treated AL"). Of these treated patients, 24 (52%) had a full or very good partial clonal

response, 16 (35%) a partial response and 6 (2%) had no response to treatment. The majority of patients were NYHA class I-II (84% AL group, 81% ATTR group). Overall, 46% of patients were in sinus rhythm, 40% in atrial fibrillation and 3% in atrial flutter. All patients had a heart rate of less than 110 beats per minute at the time of CMR scan. Median time from CMR to cardiac biopsy was 72 days.

Sixty-five (65%) of AL and 163 (88%) of ATTR patients had definite cardiac amyloidosis. Compared to patients with cardiac AL amyloidosis, patients with cardiac ATTR amyloidosis had higher LV mass index (137±33g/1.73m^2 vs 101±35g/1.73m^2, p<0.001), lower EF (54±13% vs 64±13%, p<0.001), and higher ECV (0.63±0.10 vs 0.51±0.10, p<0.001)(**Figure 1**). There were no significant differences in native T1 or serum NT-proBNP.

T2 mapping

T2 was increased in amyloidosis (AL and ATTR) compared to healthy volunteers and highest in treatment naïve AL patients (AL untreated 56.6±5.1ms; AL treated 53.6±3.9ms; ATTR 54.2±4.1ms; controls 48.9±2.0ms; p<0.01 for all pairwise comparisons except AL treated vs ATTR) (**Figure 2a**). These differences remained present after exclusion of patients with no cardiac amyloid and TTR mutation carriers (AL untreated 58.5±5.5ms; AL treated 55.1±5.5ms; ATTR 54.5±4.1ms; controls 48.9±2.0ms; p<0.05 for all pairwise comparisons except AL treated vs ATTR). ECV was highest in ATTR amyloidosis (AL untreated 0.48±0.13; AL treated 0.42±0.10; ATTR 0.60±0.13, 0.28±0.03; controls; p<0.01 for all pairwise comparisons except AL untreated vs AL treated)**(Figure 2b)**. In the overall population, and separately in AL and ATTR, there was weak association between T2 and markers of disease severity including native T1, ECV, extent of LGE, LV mass index and NT-proBNP, and no correlation with New York Heart Association (NYHA) class, heart rate or C-reactive protein (**Table 2**).

At follow up (mean 22.8±14.7 months), 75 (26%) of 286 patients had died, 28 (28%) in the AL group and 47 (25%) in the ATTR group. In AL amyloidosis, survival curves indicate that at 18 months there was approximately 88% chance of survival if T2 was less than 55ms compared to 67% if T2 was more than 55ms (p=0.01)(**Figure 3**), whilst in ATTR amyloidosis there was no relationship between T2 and prognosis (p=0.126). Using Cox regression models, T2 predicted mortality in AL amyloidosis (hazard ratio (HR) 1.48, 95% confidence interval (CI) 1.20-1.82, p<0.001) and remained significant after adjusting for ECV and NT-proBNP (HR 1.32, 95% CI 1.05-1.67, p<0.05)**(Table 3)**. ECV and NT-proBNP also remained independently predictive of mortality. After removal of patients with no cardiac involvement, the model remained predictive (HR 1.37, 95% CI 1.10-1.70, p<0.05; HR after adjusting for ECV and NT-proBNP 1.35, 95% CI 1.04-1.74, p<0.05). Alternative models were explored and T2 remained predictive of outcome after adjusting for NYHA class and E/e' (HR 1.34, 95% CI 1.09-1.64, p<0.01), LV ejection fraction and LV mass (HR 1.44, 95% CI 1.16-1.80, p=0.001) and LV ejection fraction and E/e' (HR 1.41, 95% CI 1.13-1.76, p<0.01). For ATTR, T2 was not predictive of mortality (HR 0.84, 95% CI 0.68-1.04, p=0.104).

Histological analysis

Of the 16 biopsy samples analyzed, 14 (87.5%) had evidence of myocardial edema on HE, defined as expansion of the interstitial space between myocytes(21,22) in the absence of fibrosis or amyloid. Increased space between myocytes was also observed with Congo red staining under bright field and fluorescent light in patients who had evidence of edema on samples stained with HE. The extent of edema was assessed by visual analysis and ranged from 5% to 40%. All samples had evidence of amyloid infiltration on Congo red staining and demonstrated apple-green birefringence under cross-polarized light. There was no correlation

between visually assessed percent edema on biopsy and myocardial T2 (r=-0.265, p=0.321). **Figures 4 and 5** show two examples of patients with high and low degrees of edema and their respective T2 maps on CMR.

All samples had presence of macrophages (CD68+) and leucocytes (CD45+), but none of the samples reached diagnostic criteria for inflammatory infiltration (≥14 infiltrating immune cells/mm^2)(19,20). It was observed that clustering of leucocytes and macrophages occurred around areas of amyloid infiltration (**Figure 6**). All of the myocarditis samples showed evidence of edema on HE, which appears similar to that seen in the amyloid samples. The myocarditis samples also showed extensive infiltration of immune cells, reaching diagnostic criteria for inflammation in all cases (**Figure 7**)

Discussion

The presence and degree of cardiac involvement in amyloidosis is the major determinant of survival(23,24). In this study, we demonstrate evidence of myocardial edema in amyloidosis on histology and using CMR myocardial T2. Patients with untreated AL amyloidosis show the greatest increase in myocardial T2. Myocardial T2 is predictive of prognosis in AL amyloidosis even when adjusted for ECV and NT-proBNP, but not in ATTR.

CMR is now considered the imaging tool of choice for diagnosis of cardiac involvement in systemic amyloidosis. Recently, interest has emerged in measuring amyloid burden using CMR to improve the risk stratification models and provide insight into pathogenesis(25-27) but a discrepancy has emerged with the degree of amyloid infiltration typically being more severe in ATTR but survival being worse in AL(5,28). This paradox suggests that additional mechanisms beyond amyloid infiltration may contribute to the greater mortality in AL amyloidosis, such as light chain toxicity, previously demonstrated in-vitro(8), or faster rate of amyloid deposition. In

the present study, we confirm this discrepancy and, with the use of histology and T2 mapping, have demonstrated evidence of myocardial edema, a possible additional mechanism of myocardial damage in AL amyloidosis. The use of T1 mapping, LGE, ECV quantification and T2 mapping allowed us to visualize and quantify both myocardial infiltration and edema, adding this latter dimension to tissue characterization that has traditionally focused solely on amyloid infiltration.

In the present study, we demonstrate T2 elevation in both types of amyloidosis. The degree of T2 elevation was highest in AL amyloidosis patients prior to initiation of chemotherapy, with treated AL patients having lower T2 levels, comparable to those found in ATTR amyloidosis. ECV and NT-proBNP are known to be independent predictors of outcome in both AL and ATTR amyloidosis(3,29). We demonstrate that additionally, T2 is also a predictor of mortality in AL amyloidosis after adjusting for ECV and NT-proBNP supporting an independent role for myocardial edema in outcomes.

Our histological analysis is the first to show high prevalence of myocardial edema in patients with cardiac amyloidosis. Edema is difficult to assess histologically, being defined as the presence of increased space between cells (21,22) due to accumulation of serous fluid. Animal studies with heart water content measured at autopsy have shown that myocardial T2 is elevated in edematous tissue (30). Acute myocarditis is a condition known to be associated with myocardial edema and inflammation. When compared to myocarditis biopsy samples, our amyloid biopsy samples show similar cellular separation (consistent with edema) but a much lower level of immune cell infiltration. Edema can be secondary to an inflammatory process, systemic or localized, but can be also present without inflammation. In line with previous in-vitro studies, we suggest that in cardiac amyloidosis edema is associated with light chain/fibril

toxicity or differing rates of amyloid deposition and not secondary to an inflammatory process(8,31). The absence of active inflammation is in keeping with previous work showing that there is rarely significant inflammation on histology or system evidence of inflammatory reaction to amyloid deposits(32). This is also consistent with the low C-reactive protein levels and minimal histological evidence of inflammation in our cohort. There was no correlation between percent edema on biopsy and CMR measured T2, and there are a number of reasons for this. There is currently no validated method to objectively quantify edema on biopsy samples and therefore assessment in this study was by visual analysis. It is known that amyloid deposition is very patchy in affected organs (33). We believe that edema distribution too will be non-uniform and it is therefore not possible to accurately correlate extent of edema within a biopsy sample taken from a small area of myocardium with T2 value measured over a much larger area, and a different location. Future studies with post-mortem whole heart assessment of water content would be required to confirm this hypothesis.

Both the elevated myocardial T2 and histology of biopsy samples suggest the presence of myocardial edema. We suggest that in cardiac amyloidosis edema is present but less prominent than infiltration, and this is consistent with the range of T2 values observed, which are lower than typically seen in myocarditis and acute myocardial infarction where edema is a much more prominent feature(9,10). These results suggest that cardiac involvement in AL amyloidosis is a spectrum characterized by variable degrees of amyloid infiltration and superimposed myocardial edema, with ECV and T2 defining separate processes that both contribute to risk (**Central Illustration, Figure 1**).

Previous work on myocardial T2 signal in cardiac amyloidosis has reported conflicting results. Two studies (35 and 36 patients respectively) reported that myocardial T2 ratio (between

myocardium and skeletal muscle) was reduced in patients with cardiac amyloidosis(13,14), whilst one study (12 patients) using T2 mapping reported no difference between patients with cardiac amyloidosis and controls (34). Several reasons could be responsible for these findings. Firstly, amyloid deposition can be present in the skeletal muscle and skeletal muscle T2 may be activity-dependent(35), introducing a possible confounder when the T2 ratio between myocardium and skeletal muscle is used. Secondly, these studies all had small patient numbers with no separate analysis of AL and ATTR patients. Third, the traditional dark blood turbo spin echo techniques as used in the first two of these studies may be prone myocardial signal variation and signal loss (36).

T2 mapping measurements may be prone to technical confounders. T2 measurement using multiple single-shot T2-prepared SSFP images(17) uses 3 recovery beats. The estimate of T2 is slightly confounded by tissue T1 for 2 reasons. Firstly, there is T1-dependenet regrowth of magnetization following T2-preparation since a linear phase encode order is employed, causing a slight T1-dependent bias(37). When we simulated this effect using our protocol, T2 falls less than 1ms for a 100ms elevation in T1. Secondly, there is incomplete magnetization using 3 recovery beats reducing measured T2 slightly as T1 increases. It has previously been demonstrated that age and heart rate have little effect on measured T2 in healthy volunteers (17). Within our cohort, there was no correlation of heart rate, age or hematocrit with T2.

While T1 is elevated in cardiac amyloidosis, the correlation between native T1 and myocardial T2 is weak. Native T1 is a measure of myocardial relaxation influenced by the extracellular and intracellular compartments, both free water and water bound to large molecules such as collagen or amyloid(38), whereas T2 is more specific for free water. We propose that the measured T1 signal in cardiac amyloidosis is a composite of amyloid burden and myocardial

edema whereas T2 is more specific for edema, with the overall population comprising a spectrum with varying degrees of amyloid infiltration and myocardial edema.

Our findings have potential implications for clinical management. Currently, patient stratification by CMR is mainly based on parameters linked to amyloid infiltration such as LGE pattern and ECV. A multiparametric mapping approach has potential to change this. T2 mapping is a non-contrast technique, which could also be applied to patients with renal failure, although further studies will need to validate the role of T2 mapping in this setting.

Further work is needed to confirm the hypothesis linking myocardial edema to light chain/AL fibril toxicity or the typically rapid rate of amyloid deposition in AL amyloidosis. Our model suggests that if light chain production is "switched off" using chemotherapy, T2 may be expected to fall and track reduction in free light chains and brain natriuretic peptide. This is supported by the fact that untreated AL patients demonstrated higher T2 levels than those established on treatment. Prospective follow-up studies assessing changes within patients during chemotherapy will be able to more fully assess this hypothesis and explore the role of T2 mapping to monitor treatment response.

A limitation of this study is that we did not have information on the causes of death, as the National Amyloidosis Centre assesses patients from throughout the UK. As a result, when patients die locally, only notification of death rather than cause of death is received. This is a single center study, with no paired scans performed before and after chemotherapy. Furthermore, the T2 values may not have the same reference range in other CMR scanners. Finally, troponin was measured only in a minority of patients and therefore not included in the final analysis.

Conclusions

Myocardial edema is present in cardiac amyloidosis as confirmed on histology and measured by CMR with T2 mapping. T2 mapping provides unique information in AL amyloidosis, with elevation associated with increased risk of death remaining an independent predictor of prognosis after adjustment for known prognostic factors. These findings support the concept of AL amyloidosis not being a disease of pure infiltration, but one in which additional mechanisms contribute to mortality; the study highlights the potential role of CMR with multiparametric mapping for evaluating this patient population.

Clinical Perspectives

Competency in medical knowledge: On histology, myocardial edema is a common finding in patients with cardiac amyloidosis. CMR T2 mapping is a useful tool for measuring myocardial edema in cardiac amyloidosis. Measurement of T2 is a useful additional tool in disease understanding and the risk stratification of patients with AL amyloidosis.

Translational Outlook: Future longitudinal studies in patients with amyloidosis could assess the role of T2 in tracking treatment response and clinical deterioration.

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Figure Legends

Central illustration: Myocardial edema in amyloidosis. In this study, cardiovascular magnetic resonance (CMR) with T2 mapping and histological analysis were used to characterize myocardial edema in amyloidosis. Edema is prevalent on endomyocardial biopsy of patients with amyloidosis. Myocardial T2 is elevated in amyloidosis compared to healthy controls with the degree of elevation being the highest in untreated AL patients. T2 is predictive of prognosis in AL amyloidosis, remaining significant when adjusted for extracellular volume (ECV) and NT-proBNP.

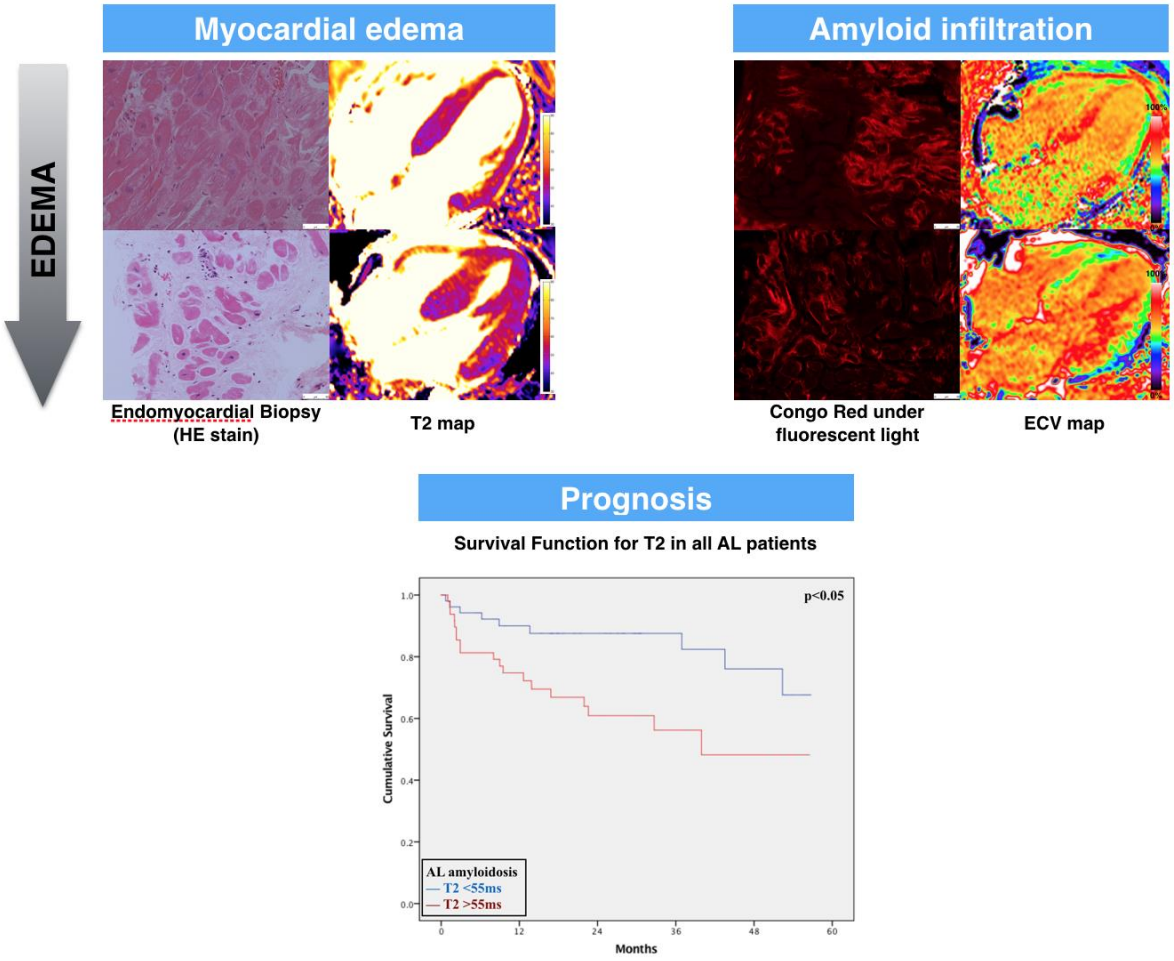

Figure 1: Characteristic CMR scans. Example CMR images of patients with cardiac AL amyloidosis (top row), cardiac ATTR amyloidosis (middle row) and a healthy volunteer (bottom row). Images (from left to right): SSFP cine at end-diastole(ED); late gadolinium(LGE) imaging(PSIR); native T1 map(MOLLI); T2 map; Extracellular volume(ECV) map.

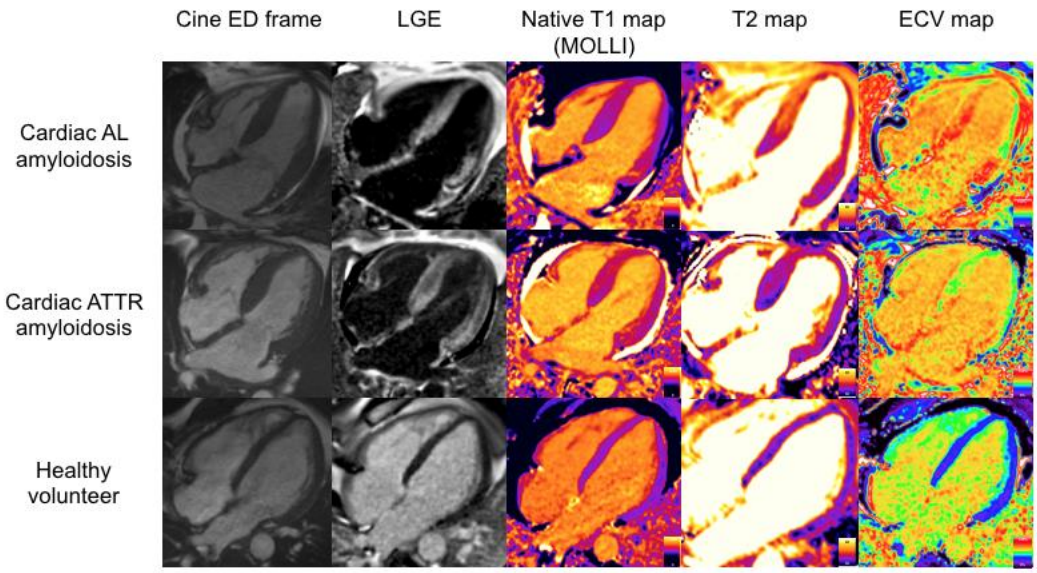

Figure 2: Mean T2 and ECV in cardiac amyloidosis and healthy volunteers. Mean T2 (± standard deviation) showing increased T2 in untreated cardiac AL amyloidosis compared to treated cardiac AL and cardiac ATTR amyloidosis(2a). Mean ECV (± standard deviation) showing increased ECV in cardiac ATTR amyloidosis compared to treated and untreated AL amyloidosis(2b).

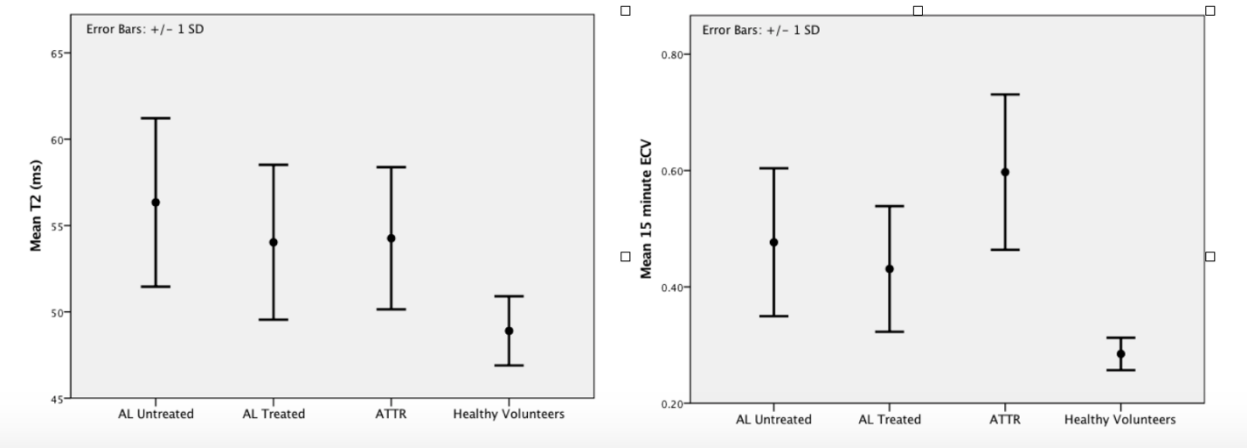

Figure 3: T2 and survival in AL amyloidosis. Kaplan-Meier survival curves for patients with AL amyloidosis with high T2 and normal T2 values.

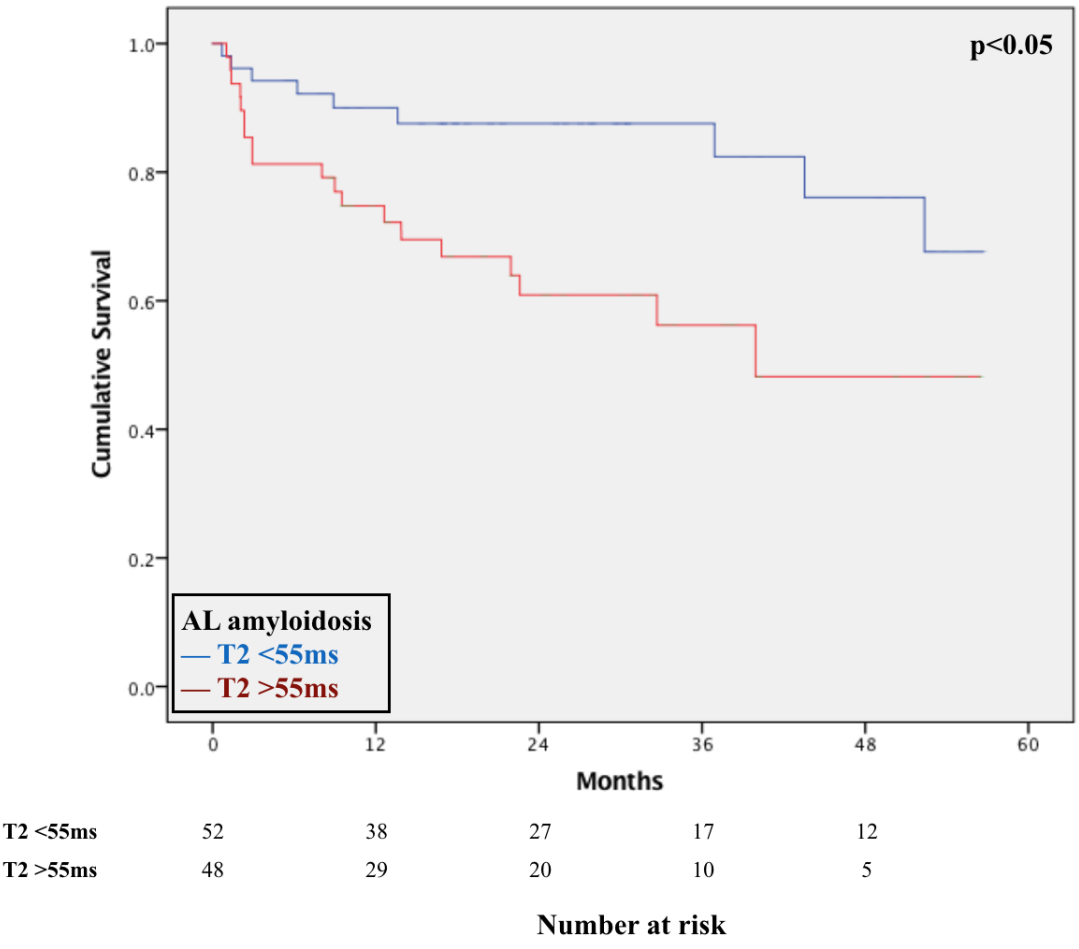

Figure 4: Myocardial biopsy and T2 map of patient with AL amyloidosis. Haemotoxylin and eosin(HE) staining shows increased space around myocytes (black arrows) consistent with edema. Congo red staining viewed under bright field(CR) and fluorescent light(FL) shows amyloid deposits (yellow arrows) and increased space between cells (white arrows). T2 map shows diffuse increased in T2 (57ms in septum).

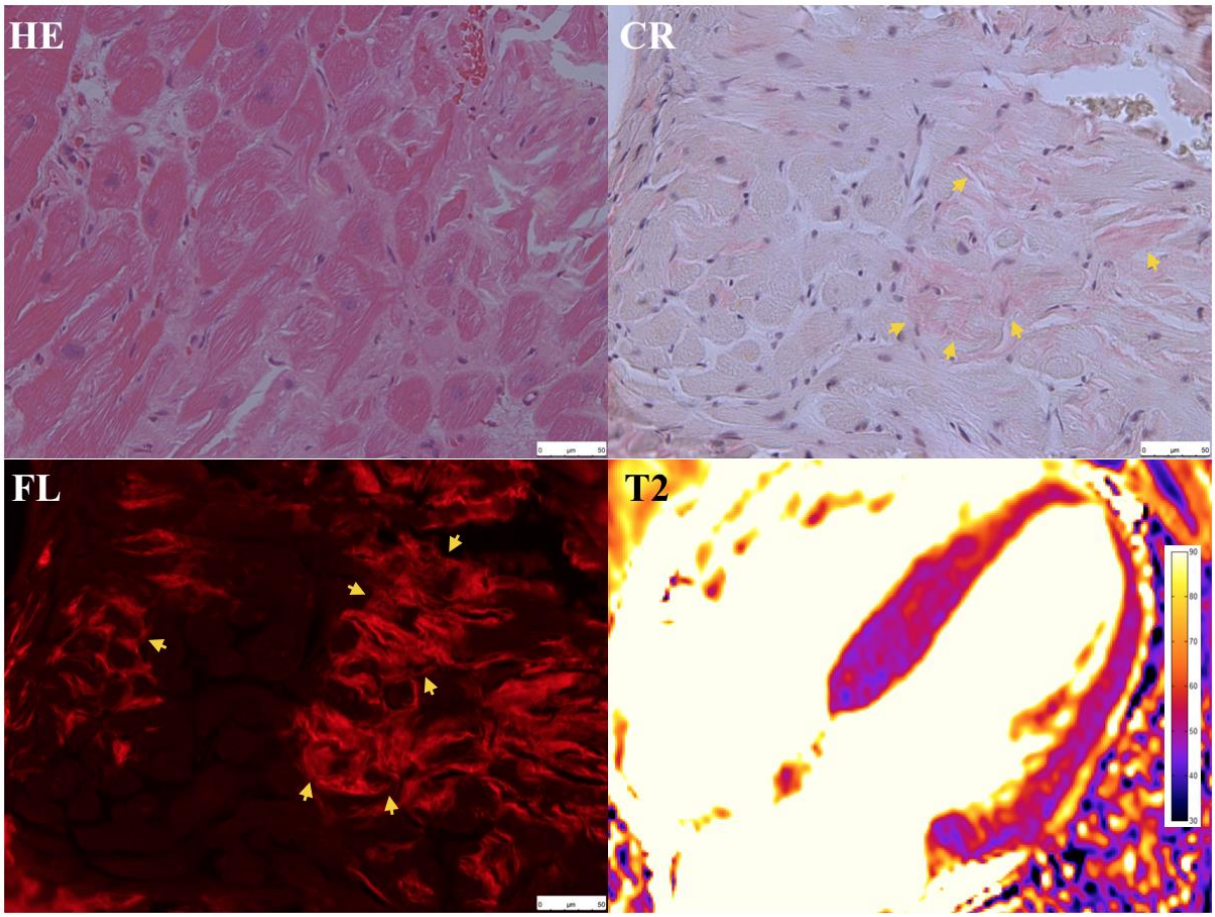

Figure 5: Myocardial biopsy and T2 map of patient with ATTR amyloidosis. Haemotoxylin and eosin(HE) staining shows no evidence of edema. Congo red staining viewed under bright field(CR) and fluorescent light(FL) shows amyloid deposits (yellow arrows). T2 map shows normal T2.

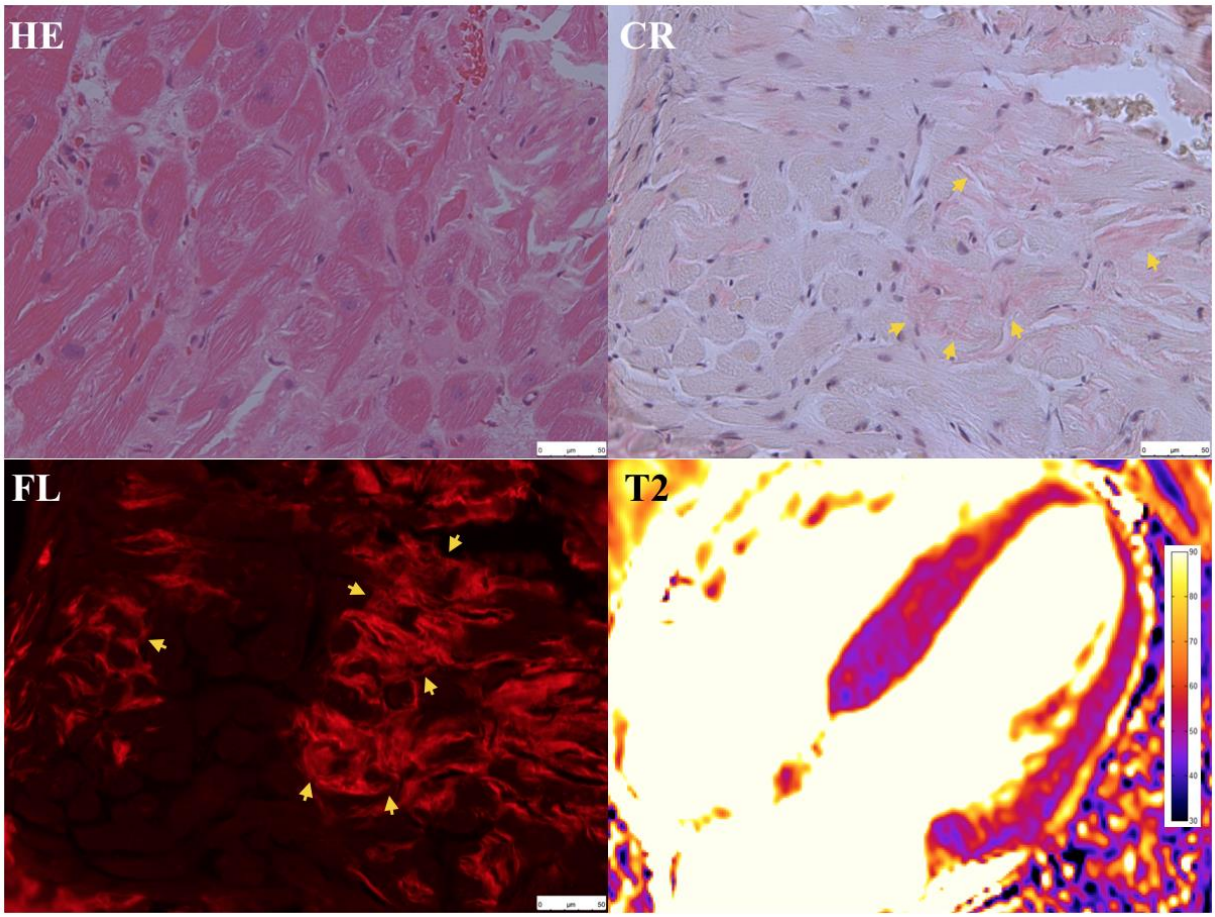

Figure 6: Example of histology images of a patient with cardiac amyloidosis. Presence of leucocytes (CD45) and macrophages (CD68) adjacent to area of amyloid infiltration (white arrows). Amyloid infiltration (yellow arrows) demonstrated on Congo red staining under bright field(CR) and polarized light(PL) demonstrating apple green birefringence.

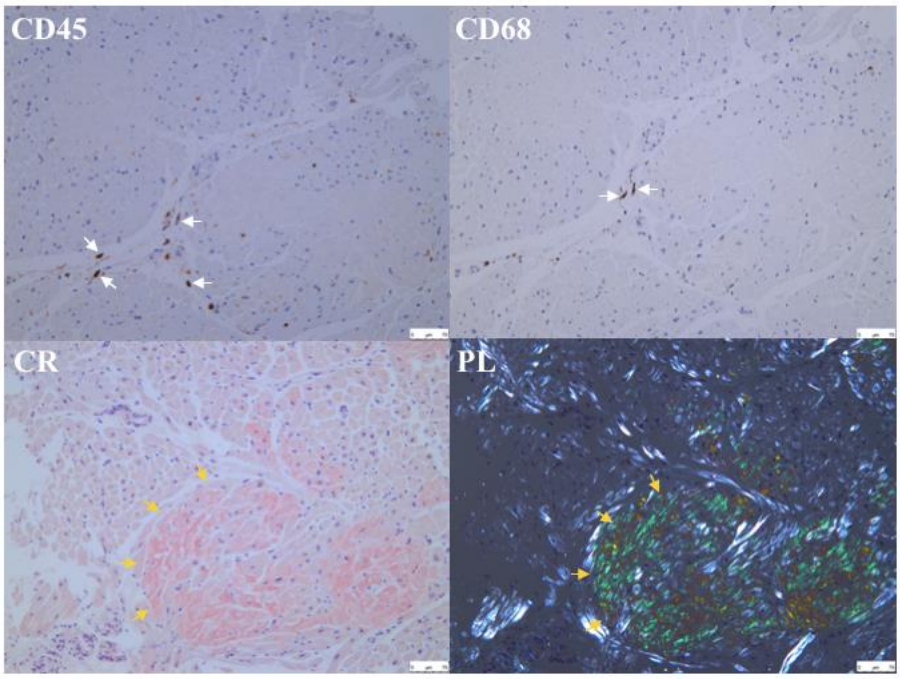

Figure 7: Comparison of cardiac amyloidosis and acute myocarditis on histology.

Myocardial biopsies of patients with cardiac amyloidosis (A) and acute myocarditis (B,C). Haemotoxylin and eosin (HE) staining shows increased space around myocytes in both amyloidosis and myocarditis (white arrows) consistent with edema. Myocarditis shows much greater infiltration of immune cells compared to amyloidosis (yellow arrows). Example C also shows an area of abscess (red arrows)

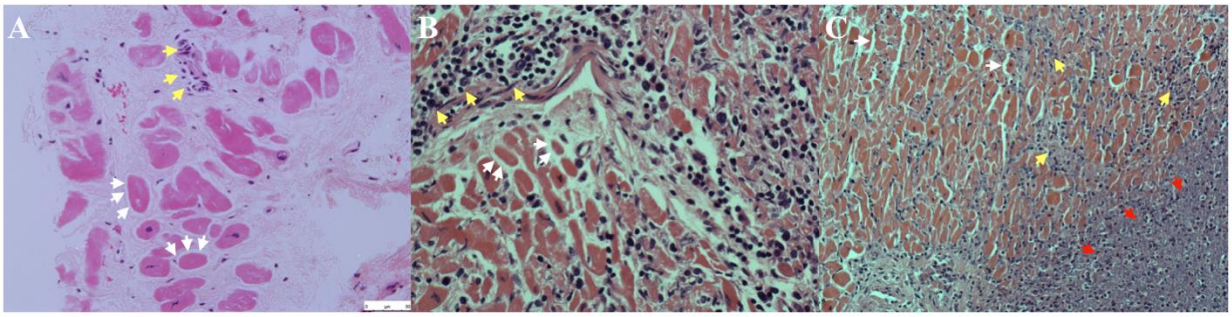

Table 1: Baseline characteristics of participants with AL amyloidosis with and without cardiac involvement, cardiac ATTR amyloidosis, possible cardiac ATTR amyloidosis and TTR gene mutation carriers

	AL amyloidosis			ATTR		
	No CA (n=35)	**CA (sub-endocardial LGE) (n=37)**	**CA (transmural LGE) (n=28)**	**TTR gene mutation carriers (n=11)**	**Possible CA (n=12)**	**Definite CA (n=163)**
Age, years	63.9±9.4	64.0±11.5	62.7±12.3	45.6±7.5	73.3±14.4	74.6±8.1†
Males, n (%)	15 (43%)	27 (73%)	19 (68%)	4 (36%)	8 (67%)	139 (85%)†
Diabetes, n(%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	15 (9%)
Hypertension,n(%)	14 (40%)	5 (14%)	1 (4%)*	0 (0%)	3 (25%)	36 (22%)
Ischaemic heart disease, n(%)	2 (6%)	8 (22%)	3 (11%)	0 (0%)	2 (17%)	21 (13%)
Systolic BP, mmHg	138±24	125±17	117±16*	118±11	142±24	122±15†
Diastolic BP, mmHg	80±10	73±12	70±11*	70±10	77±15	73±11
Heart rate, beats per minute	75±12	76±14	84±14*	70±11	74±11	69±12

6 minute walk distance, m	366±125	353±105	362±173	511±42	372±175	283±135†
NYHA class, n (%)						
I	17(49)	15(41)	7(25)	11(100)	7(58)	19(12)
II	16(46)	20(54)	10(36)	0(0)	4(33)	110(68)
III	2(6)	2(5)	11(39)	0(0)	1(8)	33(20)
IV	0(0)	0(0)	0(0)	0(0)	0(0)	1(1)
Echo parameters						
E/A	0.9±0.4	1.5±1.1	1.6±1.0*	1.2±0.3	1.1±0.3	3.8±10.3
E/e'	10.1±5.4	13.8±7.8	16.6±9.4*	13.1±18.7	8.5±2.8	17.1±6.3†
CMR parameters						
LVEDVi, ml/m^2	70±21	66±17	61±16	66±13	66±14	71±19
LVESVi, ml/m^2	23±15	22±13	26±13	22±6	25±16	33±16†
LVEF, %	68±9	67±12	59±13*	67±4	65±16	54±13†
SVi, ml/m^2	46±10	43±10	35±11*	44±8	42±10	38±9†
LV mass index, g/1.73m^2	79±28	92±30	113±36*	58±11	68±23	137±33†
Pre-contrast myocardial T1, ms	1007±58	1086±50	1144±75*	969±42	1003±53	1104±46†

Post-contrast myocardial T1, ms	610±53	566±127	565±64	574±45	544±58	467±57†
ECV	0.35±0.09	0.47±0.08	0.55±0.10 *	0.30±0.03	0.39±0.14	0.63±0.09 †
T2, ms	53.2±3.6	56.3±4.8	56.2±5.4*	50.4±3.2	51.5±3.7	54.7±4.0†
Hematocrit	0.370±0.0	0.374±0.0	0.400±0.0	0.421±0.0	0.404±0.0	0.408±0.0
NT-proBNP, pmol/L	37 (15-235)	162 (89-525)	241 (156-895)*	6 (3-9)	21 (10-158)	329 (177-589)†
C-reactive protein, mg/L	3.0 (1.0-5.0)	2.0 (0.5-7.0)	3.5 (1.0-8.0)	1.0 (0.3-3.0)	1.0 (0.3-3.0)	3.0 (1.0-5.0)

AL, light-chain amyloidosis; ATTR, transthyretin amyloidosis; CA, cardiac amyloidosis; CRP, C-reactive protein; CMR, cardiovascular magnetic resonance; ECV, extra-cellular volume fraction; EDVi, end diastolic volume indexed; ESVi end systolic volume indexed; LGE, late gadolinium enhancement; LV, left ventricular; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; NT-proBNP, N-terminal pro-brain natriuretic peptide; SVi, stroke volume indexed. p-values: *$p<0.05$ for trend in AL patients across different patterns of cardiac involvement. †$p<0.05$ for trend in ATTR patients across different patterns of cardiac involvement. All continuous variables are presented as mean and standard deviation with non-transformed NT-proBNP and CRP presented as median and interquartile range.

Table 2: Correlation between T2 (in ms) and cardiac function, biomarkers and 6-minute walk test

	All AL patients: correlation coefficients	All ATTR patients: correlation coefficients
LV structure and function by CMR		
LV mass index, g/m^2	0.214*	0.344†
LA area index, cm^2/m^2	0.098	0.130
LVEF, %	0.029	-0.151*
SVi, ml/m^2	-0.055	0.039
Echo E/e'	0.221*	0.151*
6-minute walk test	-0.073	-0.095
NYHA class	0.016	0.128
Heart rate, beats per minute	-0.076	-0.246*
Age, years	0.029	0.119
Blood biomarkers		
NT-proBNP, pmol/L	0.372†	0.236†
C-reactive protein, mg/L	-0.023	-0.080
Hematocrit	-0.176	-0.004
Tissue characterization by CMR		
Native T1, ms	0.439†	0.489†
ECV	0.280†	0.316†
LGE pattern	0.283†	0.285†

DPD grade	--	0.150*

Pearson correlation coefficient (Spearman for NYHA class, LGE pattern and DPD grade): *p<0.05, †<0.01 AL, light-chain amyloidosis; ATTR, transthyretin amyloidosis; CMR, cardiovascular magnetic resonance; DPD, ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid; ECV, extracellular volume fraction; LA, left atrial; LGE, late gadolinium enhancement; NYHA, New York Heart Association; NT-proBNP, N-terminal pro-brain natriuretic peptide.

Table 3: Cox proportional hazards regression analysis of predictors of mortality in AL amyloidosis

	Univariable analysis		Multivariable Analysis	
	Hazard Ratio	**p value**	**Hazard Ratio**	**p value**
Myocardial T2 (per 3ms change)	1.48 (1.20-1.82)	<0.001	1.32 (1.05-1.67)	0.02
ECV (per 0.03 unit change)	1.22 (1.10-1.37)	<0.001	1.21 (1.08-1.37)	<0.01
NT-proBNP (per 100pmol/L change)	1.02 (1.00-1.04)	0.01	1.03 (1.01-1.05)	0.01

ECV, extracellular volume fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide.

(Multivariable analysis includes each of the variables stated in the table)