

Quantification of Myocardial Extracellular Volume Fraction in Systemic AL Amyloidosis

An Equilibrium Contrast Cardiovascular Magnetic Resonance Study

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Background—Cardiac involvement predicts outcome in systemic AL amyloidosis and influences therapeutic options. Current methods of cardiac assessment do not quantify myocardial amyloid burden. We used equilibrium contrast cardiovascular magnetic resonance (EQ-CMR) to quantify the cardiac interstitial compartment, measured as myocardial extracellular volume (ECV) fraction, hypothesizing it would reflect amyloid burden.

Methods and Results—Sixty patients with systemic AL amyloidosis (65% men, median age 65 years) underwent conventional clinical cardiovascular magnetic resonance, including late enhancement, equilibrium contrast cardiovascular magnetic resonance, and clinical cardiac evaluation, including ECG, echocardiography, assays of N-terminal pro-brain natriuretic peptide and Troponin T, and functional assessment comprising the 6-minute walk test in ambulant individuals. Cardiac involvement in the amyloidosis patients was categorized as definite, probable, or none, suspected by conventional criteria. Findings were compared with 82 healthy controls. Mean ECV was significantly greater in patients than healthy controls (0.25 versus 0.40, $P<0.001$) and correlated with conventional criteria for characterizing the presence of cardiac involvement, the categories of none, probable, definite corresponding to ECV of 0.276 versus 0.342 versus 0.488, respectively ($P<0.001$). ECV was correlated with cardiac parameters by echocardiography (eg, Tissue Doppler Imaging [TDI] S-wave $R=0.52$, $P<0.001$) and conventional cardiovascular magnetic resonance (eg, indexed left ventricular mass $R=0.56$, $P<0.001$). There were also significant correlations with N-terminal pro-brain natriuretic peptide ($R=0.69$, $P<0.001$) and Troponin T ($R=0.53$, $P=0.006$). ECV was associated with smaller QRS voltages ($R=0.57$, $P<0.001$) and correlated with poorer performance in the 6-minute walk test ($R=0.36$, $P=0.03$).

Conclusions—Myocardial ECV measurement has potential to become the first noninvasive test to quantify cardiac amyloid burden. (*Circ Cardiovasc Imaging*. 2013;6:34-39.)

Key Words: amyloid ■ cardiac ■ cardiac MRI ■ cardiomyopathy ■ heart failure

Systemic AL amyloidosis is caused by the accumulation of amyloid deposits derived from monoclonal immunoglobulin light chains in the interstitium of organs throughout the body. It is the most common and serious type of amyloidosis, with treatment comprising chemotherapy directed at the underlying plasma cell dyscrasia. Cardiac involvement is frequent, a principal driver of prognosis, and can be the presenting feature of the disease.¹ Although a constellation of ECG, echocardiographic and biomarker findings becomes increasingly diagnostic and prognostic as cardiac amyloidosis progresses, evaluation of early stage cardiac involvement can be challenging. Confounding features are often present,

commonly including left ventricular (LV) hypertrophy and abnormal diastolic function associated with renal failure, diabetes mellitus, or hypertension.²⁻⁵ Definitive diagnosis of cardiac amyloidosis, which has critical implications for choice of chemotherapy and management generally requires cardiac biopsy which is invasive and prone to sampling error. There are currently no noninvasive tests that can quantify cardiac amyloid deposits, the need for this having lately intensified with development of novel anti-amyloid therapies that will shortly need testing and validation.⁶

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New imaging modalities are showing promise. Scintigraphy with technetium-labeled 3,3-diphosphono-1,2-propanodicarboxylic acid bone scanning agent (^{99m}Tc -DPD) seems to image cardiac amyloid, although chiefly transthyretin type,⁷ whereas imaging with cardiovascular magnetic resonance (CMR) is virtually pathognomonic of amyloid in some patients when characteristic global, subendocardial late gadolinium enhancement (LGE) occurs.⁸ Neither is quantitative. We have lately developed a new quantitative technique, equilibrium contrast CMR (EQ-CMR) that measures the myocardial extracellular volume (ECV) fraction, ie, the interstitial space within the heart, which is expanded by fibrosis in many types of cardiac disease. Here, we hypothesize that the technique would also be an effective method for quantifying cardiac amyloid burden, the proteotypic interstitial protein deposition disorder.⁹

Methods

Cardiovascular Magnetic Resonance

The research received local ethical approval and all participants provided informed consent. EQ-CMR uses a conventional magnetic resonance imaging scanner and is an add-on to a standard clinical scan. The technique allows quantification of the myocardial volume of distribution of the routine clinical contrast agent, Gadoterate meglumine (gadolinium-DOTA, marketed as Dotarem Guerbet S.A. France), which partitions freely between the plasma and interstitial space but does not enter cells. Interstitial tissue volume is primarily determined by the amount of extracellular matrix. EQ-CMR involves 3 steps: (1) a standard gadolinium bolus followed by constant infusion to eliminate contrast kinetic effects and achieve an equilibrium contrast state throughout the body; (2) signal intensity (T1) measurement pre- and postcontrast equilibrium using CMR; and (3) a direct measure of blood volume of contrast distribution, by taking a complete blood count, equating the blood volume of contrast distribution to 1 minus the hematocrit. The volume of distribution in the myocardium, also known as ECV, is then calculated as follows:

$$\text{ECV} = (1 - \text{Hematocrit}) \times (\Delta R1_{\text{myocardium}} / \Delta R1_{\text{blood}}) \quad (1)$$

where $\Delta R1$ is $(1/T_1 \text{ precontrast} - 1/T_1 \text{ postcontrast})$

EQ-CMR was performed on a 1.5-T magnet (Avanto, Siemens, 16 channel coils) with T1 assessment as previously described.¹⁰

Patients

Sixty consecutive consenting patients with systemic (primary) AL amyloidosis who were assessed between 2010 and 2011 at the National Amyloidosis Center (Royal Free Hospital, London, United Kingdom) and in whom there were no exclusions to CMR (glomerular filtration rate <30 mL/min; presence of nonmagnetic resonance compatible devices, known atrial fibrillation) were recruited. Approximately, 25% of patients with systemic AL amyloidosis seen at the center during this period had an estimated glomerular filtration rate of <30 mL/min per 1.73 m² and were therefore excluded. Four patients who were found to have atrial fibrillation/flutter after they had consented were not excluded. Of this cohort, 7 patients (12%) were on treatment for hypertension, and 6 (10%) had confirmed coronary artery disease by angiography. Patient characteristics are described in Table 1. All patients were required to have histological proof of systemic AL amyloidosis by Congo red histology and immunohistochemical staining, which was obtained through specimens of kidney (n=17, 28%), endomyocardium (n=4, 6%), bone marrow (n=8, 13%), upper gastrointestinal tract (n=4, 6%), liver (n=1, 2%), fat (n=6, 10%), spleen (n=1, 2%), lung (n=1, 2%), rectum (n=8, 13%), soft tissues (n=8, 13% included skin, tongue, buccal mucosa, and labia), lymph node (n=1, 2%), and peritoneum (n=1, 2%).

Healthy subjects (n=82) were recruited through advertising in hospital, University, and general practitioner surgeries. All had no history or symptoms of cardiovascular disease or diabetes mellitus, a normal 12-lead ECG and normal clinical CMR scan. No patient was on

Table 1. Baseline Characteristics of Patients with AL and Healthy Controls

Characteristic	Patients	Healthy Controls	P
Men/women	39/21	41/41	0.08
Mean age \pm SD, y	62 \pm 10	46 \pm 15	<0.001
Mean creatinine \pm SD, mmol/L	84 \pm 26	74 \pm 13	0.006
Median NT-proBNP, pmol/L (interquartile range)	121 (20–272)	...	
Organ involvement			
Peripheral/autonomic neuropathy, %	14 (23)	...	
Renal involvement, %	30 (50)	...	
Cardiac involvement			
None, %	15 (25)	...	
Probable, %	15 (25)	...	
Definite, %	30 (50)	...	
Echocardiography/CMR indices			
Mean indexed end-diastolic LV volume \pm SD, mL	60 \pm 13	72 \pm 12	<0.001
Mean indexed end-systolic volume \pm SD, mL	19 \pm 8	23 \pm 7	<0.001
Mean EF \pm SD, %	67 \pm 11	67 \pm 5	0.44
Mean indexed LV mass \pm SD, g/m ²	94 \pm 34	66 \pm 13	<0.001
AF/atrial flutter, %	4 (7)	0	

AF indicates atrial fibrillation; AL, light-chain amyloidosis; CMR, cardiovascular magnetic resonance; EF, ejection fraction; LV, left ventricular; and NT-proBNP, N-terminal pro-brain natriuretic peptide.

cardioactive medication except 4 on statins for primary prevention. The baseline characteristics of the healthy controls are provided in Table 1.

All patients and healthy controls underwent 12-lead ECG. Patients additionally underwent assays of cardiac biomarkers (N-terminal pro-brain natriuretic peptide [NT-proBNP] and Troponin T), echocardiography, SAP scintigraphy, including measurement of total body amyloid load, and a 6-minute walk test, although this was not possible in some patients (n=23, 38%) mainly because of other comorbid factors (eg, arthritis, postural hypotension, peripheral neuropathy) and patient choice. New York Heart Association class and Eastern Cooperative Oncology Group status were also assessed using a standard questionnaire. All ethics were approved by the UCL/UCLH Joint Committees on the Ethics of Human Research Committee.

Analysis

Standard CMR parameters of structure (LV mass, left atrial area with/without indexing, septal thickness), systolic function (ejection fraction, mitral annular plane systolic excursion [MAPSE] and tricuspid annular plane systolic excursion [TAPSE]) as well as ECV calculation were assessed. A region of interest was drawn in the basal septum in a piloted 4-chamber view in all patients; regions of interest were mid myocardial and excluded subendocardial LGE. Where gadolinium was present, it was often extensive and therefore impossible to exclude from the region of interest (Figure 1). Mean ECG QRS voltage in limb and precordial leads were calculated.¹¹ Echocardiographic assessment of diastolic function was performed (transmitral E-wave deceleration time, intraventricular relaxation time, E:E' ratio).

Statistical analysis was performed using SPSS (IBM version 20). Normal distribution was assessed using the Kolmogorov–Smirnov test. All variables were normally distributed, other than NT-proBNP which was therefore log transformed for bivariate testing. Bivariate analysis compared ECV with all other variables. Pearson correlations are presented in terms of R values unless otherwise stated. χ^2 test was used to compare LGE positive and negative groups, and the unpaired *t* test was used to compare continuous data between groups. An 1-way ANOVA analysis was used to assess the correlation of ECV with New York Heart Association class and Eastern Cooperative Oncology

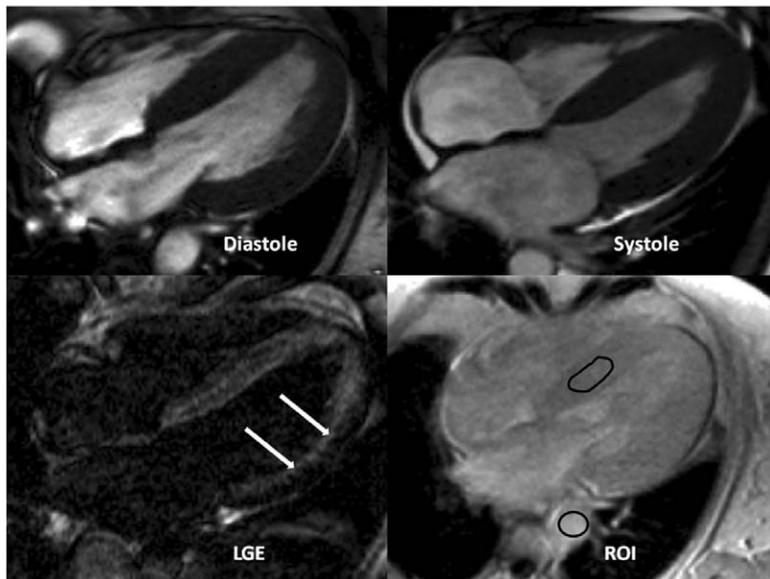


Figure 1. Four-chamber cine image in diastole and systole (top); with late gadolinium enhancement (LGE) image and single-slice, precontrast FLASH T1 image at a TI of 200 ms (bottom), 1 of 7 frames showing regions of interest (ROI) in basal septum and descending aorta.

Group status. Means \pm SD are presented. The Bonferroni correction was used to test ECV against pretest probability for the presence of cardiac involvement based on clinical assessment. The probability of cardiac involvement was categorized into definite or no cardiac involvement based on international consensus criteria published by Gertz et al.¹² An additional category of possible involvement was created for patients with cardiac abnormalities in whom there were confounding features. The categorization was defined as follows:

Definite cardiac involvement, any of

- LV wall thickness of ≥ 12 mm by echocardiography in the absence of any other known cause;
- Right ventricular free wall thickening coexisting with LV thickening by echocardiography in the absence of systemic or pulmonary hypertension.

Possible cardiac involvement, any of

- LV wall thickening by echocardiography in the presence of hypertension;
- Right ventricular thickening by echocardiography in the presence of pulmonary hypertension;
- Normal wall thickness by echocardiography with diastolic dysfunction and raised serum biomarkers.¹³

No suspected involvement

- Normal wall thickness by echocardiography with normal serum biomarkers.

For interobserver reproducibility, scans were analyzed independently (S.B., V.M.) and the results were considered as a Bland–Altman plot and were analyzed using intraclass correlation coefficient. For interstudy reproducibility, patients and healthy volunteers reattended scanning for a minimum of 5 and a maximum of 14 days apart for repeat EQ-CMR. Analysis was as above: S.B. analyzed both after anonymization on separate days with results collated by V.M.

Results

Baseline patient and healthy control characteristics are shown in the Table 1 and summary of correlations in Table 2. The mean ECV in patients with systemic amyloidosis was significantly elevated compared with healthy controls (0.400 versus 0.254, $P < 0.001$). Mean ECV increased between groups ($P < 0.001$) from healthy controls to AL with no suspected cardiac involvement (mean ECV 0.276), possible cardiac involvement (mean ECV 0.342), and definite cardiac involvement (mean

ECV 0.488) (Figure 2A and 2B). There was no statistically significant increase in ECV with age in the healthy controls.

LGE prevalence overall was 35 of 60 (58%). The pattern of LGE was global, subendocardial ($n=21$), patchy ($n=7$), and extensive ($n=7$) (Figure 3). There was no significant difference in the mean ECVs between these groups. LGE presence was strongly tied to the pre-CMR likelihood of cardiac involvement: no, possible, definite cardiac involvement LGE prevalences were 0 of 15 (0%), 6 of 15 (40%), and 29 of 30 (99%), respectively ($P < 0.001$).

ECV correlated strongly with the presence of LGE ($P < 0.001$). All patients with LGE had an elevated ECV. In 4 cases, sufficient LGE negative areas existed to permit additional LGE negative ROIs to be drawn. In these cases, the ECV was lower than that derived from adjacent LGE areas but still demonstrated substantial elevation (0.45, 0.44, 0.37, and 0.50 compared with 0.46, 0.50, 0.44, and 0.53, respectively). Of the 25 patients without LGE, 6 (24%) had elevated ECV, suggesting earlier detection using ECV measurements.

ECV was correlated with CMR cardiac parameters of LV mass, septal thickness, size (end-systolic volume [ESV] and indexed End-Systolic Volume [ESVi]), and left atrial area; measures of cardiac function (CMR and echocardiography), including both simple (MAPSE and TAPSE); and advanced parameters (TDI S-wave, E/E', Table 2). Hypertrophy was not necessary for ECV elevation; 4 patients without hypertrophy had raised ECVs (0.34, 0.35, 0.42 and 0.52).

ECV correlated with the biomarkers NT-proBNP and Troponin T (Table 2). ECV was inversely correlated with mean QRS voltage, particularly in the limb leads (limb leads, $R=0.57$, $P < 0.001$; precordial leads, $R=0.30$, $P=0.02$) (Table 2 and Figure 4).

Performance in the 6-minute walk test among patients with possible and definite cardiac involvement by conventional assessment was poorer than for those with no suspected cardiac involvement (218 versus 207 versus 265 m, both $P=0.01$). ECV weakly correlated with 6-minute walk test ($R=0.36$, $P=0.03$). There were no differences of ECV with New York Heart Association class or Eastern Cooperative Oncology Group class.

Table 2. ECV Correlations With Cardiac Structure and Function, Biomarkers, and ECG Changes in Patients

ECV Compared Against	<i>R</i>	<i>P</i>
LV Structure by CMR		
LV mass	0.52	<0.001
Indexed LV mass	0.56	<0.001
Septal thickness	0.56	<0.001
LA area	0.40	0.002
Indexed LA area	0.49	<0.001
LV systolic function by CMR		
Ejection fraction	0.58	<0.001
MAPSE	0.58	<0.001
LV end-systolic volume	0.52	<0.001
Indexed LV end-systolic volume	0.56	<0.001
TDI-S wave	0.52	<0.001
LV diastolic function by echo		
E:E'	0.47	<0.001
IVRT	0.36	0.03
E-deceleration time	0.48	<0.001
RV structure and function by CMR		
RV end-systolic thickness	0.40	0.005
TAPSE	0.50	<0.001
Functional assessment		
6-minute walk test	0.36	0.03
Biomarkers		
Serum NT-pro BNP (log BNP)	0.69	<0.001
Troponin T	0.53	0.006
ECG		
ECG limb lead mean voltage	0.57	<0.001
ECG chest lead mean voltage	0.30	0.02

CMR indicates cardiovascular magnetic resonance; ECV, extracellular volume fraction; IVRT, isovolumic relaxation time; LA, left atrial; LV, left ventricular; MAPSE, mitral annular plane systolic excursion; NT-proBNP, N-terminal pro-brain natriuretic peptide; RV, right ventricular; TAPSE, tricuspid annular plane systolic excursion; and TDI, tissue Doppler imaging.

Intrastudy, interobserver, and interstudy reproducibility of the EQ-CMR technique was tested in $n=7$ patients (5 with AL and 2 with transthyretin amyloid). All interstudy scans were performed within a 14-day period. Intrastudy correlation coefficient was 0.99 (0.96–0.99) with a bias of 0.002 (–0.009 to 0.014). The intraclass correlation coefficient (ICC) for interobserver reproducibility was 0.92 (0.76–0.98) with a bias of 0.01 (–0.01 to 0.04) and for interstudy reproducibility was 0.96 (0.90–0.99) with a bias of 0.02 (–0.03 to 0.07).

Discussion

Amyloidosis is the exemplar of an interstitial disease, the quantity of amyloid in the extracellular space amounting to kilograms in some patients. Cardiac involvement is a major cause of morbidity and mortality, particularly in AL type, but there are currently no noninvasive methods to quantify it. Here, the ECV was measured using EQ-CMR in systemic AL amyloidosis. ECV was massively elevated in the patients with definite cardiac involvement but also significantly higher in patients where conventional clinical testing suggested no cardiac involvement and

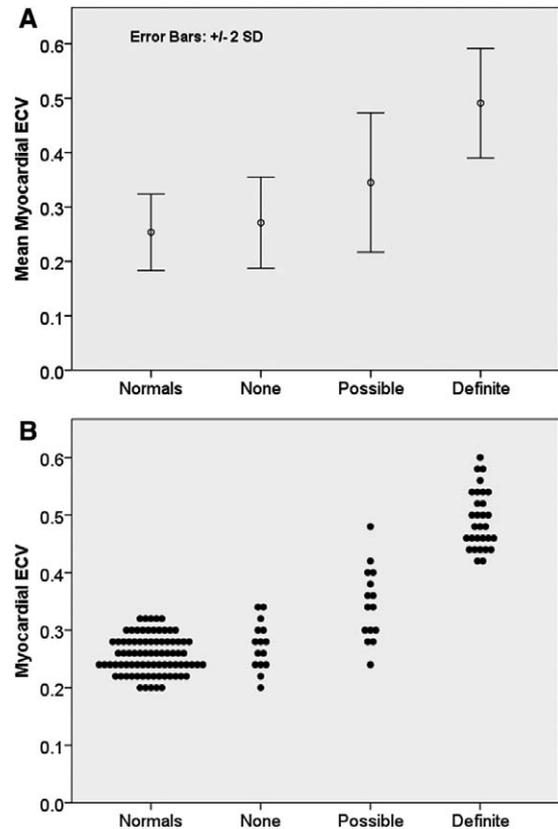


Figure 2. (A) Mean extracellular volume fraction (ECV ± 2 SD) and (B) dot plot showing individual results in bins of 0.02 (0.20–0.60) in healthy controls subjects vs systemic AL amyloidosis patients with 3 grades of cardiac involvement by conventional testing: no suspected involvement, possible, and definite involvement.

was significantly elevated in a quarter of patients with no LGE. ECV tracked a wide variety of markers of disease activity, such as cardiac function and blood biomarkers, linked to patient's functional performance, and strongly correlated with limb lead ECG complex sizes. These data suggest that ECV measurement is picking up infiltration earlier than conventional testing and is a direct measure of the amyloid burden with potential use in early diagnosis, disease monitoring, and, potentially, as a much needed cardiac surrogate end point for the various promising new therapies of amyloidosis currently in preclinical development and early phase clinical trials.^{6,14}

Expansion of the myocardial ECV represents a nonspecific increase in free water in myocytes and occurs in a variety of pathologies, including focal and diffuse fibrosis, edema, and amyloidosis. However, the ECV in amyloidosis is higher than in any other disease-generating diagnostic specificity above a certain threshold, for example, in our previous work ($n=238$) the highest ECV we have found to date in severe aortic stenosis, dilated or hypertrophic cardiomyopathy, was 0.37 in a severe aortic stenosis patient with previous coarctation repair and a poor LV.¹⁵ Here, the average value for definite cardiac AL amyloid was 0.488 with the lowest value being 0.423. We note, however, that the amyloid ROIs frequently included a proportion of areas of LGE, which was excluded in the previous study.

A limitation of this study is the lack of histological correlations. In previous work, we have correlated diffuse fibrosis with ECV, but only 4 patients in our present cohort had undergone cardiac biopsy, and only 1 of these was performed

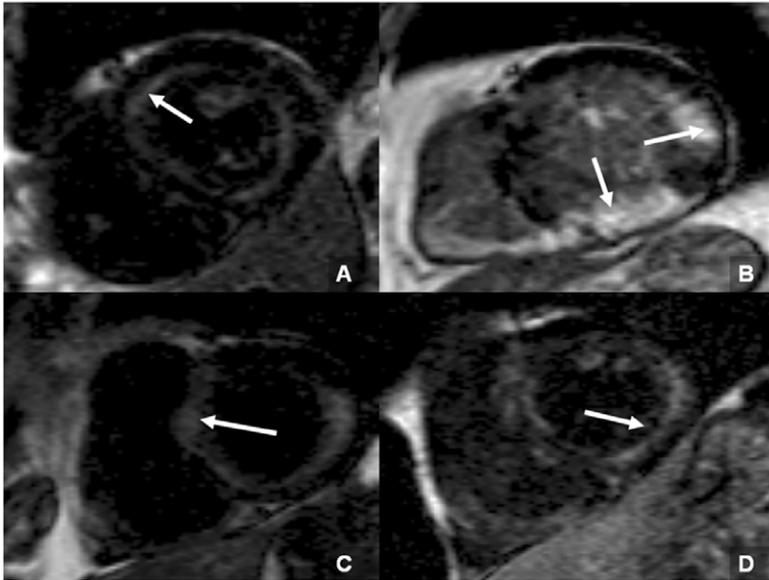


Figure 3. Late gadolinium enhancement (LGE) patterns: characteristic global, subendocardial (A); patchy (B); extensive (C), and (D) LGE in a patient without hypertrophy (maximum wall thickness in this individual was 10 mm).

within 4 months of the CMR study. Precise calibration of ECV with histological estimation of amyloid burden is desirable but challenging because, often in this multisystem disease, other organs are biopsied before cardiac involvement is recognized and other organs are easier to sample. Furthermore, amyloid is patchy predisposing to biopsy sampling error. We estimate that 25 to 30 biopsy samples would be required for ECV calibration, a work in process.

In this study, strong correlations of ECV were also seen with indexed LV mass, left atrial area, septal thickness and CMR markers of LV systolic function, and more modest correlations with measurements of diastolic function, although when systolic function was measured by echo, correlations for diastolic and systolic function were broadly at the same strength. The correlations seem to support many conventional clinical strategies, for example, that indexed left atrial area as an excellent indicator of ventricular disease, the use of limb lead QRS voltages and the use of NT-proBNP and Troponin T in the Mayo clinical staging system.¹³ However, biomarker experience shows that single time point ECV measurement is not the whole picture - post chemotherapy, light chain production switch off may trigger a swift change in NT-proBNP levels (usually falls, sometimes elevation)^{4,16} without probable ECV changes (no ECG or echo changes), suggesting that simple amyloid burden quantification incompletely reflects myocardial pathology in amyloid.

Mean limb rather than precordial lead QRS voltage on ECG showed an inverse correlation with ECV. Approximately 50% of patients with cardiac AL amyloidosis have low QRS voltage (<6 mm),¹⁷ which has been attributed to insulation of the electric impulse on the surface ECG owing to infiltration. However, it is not seen in all patients and increased QRS voltages sometimes occur.¹⁸ Cardiac transthyretin amyloidosis may have far more wall thickening than AL amyloid yet, low voltages may not occur. Wall thickening in amyloid is the composite of myocyte volume and amyloid burden, with myocyte volume the balance between myocyte death^{3,19} and hypertrophy.²⁰ Conventional testing (such as LV mass) measure the composite of infiltration and cell volume. Further study with EQ-CMR histological calibration to derive myocyte volume as well as ECV will be able to answer important questions about the biology of cardiac amyloid.

A significant correlation with ECV was seen with performance in the 6-minute walk test but not with New York Heart Association and Eastern Cooperative Oncology Group performance status, although walking ability in patients with systemic amyloidosis can be influenced by many noncardiac factors, including peripheral and autonomic neuropathy, peripheral edema, pleural effusions attributed to nephrotic syndrome, bone pain attributed to myeloma, and skeletal muscle loss associated with malnutrition.

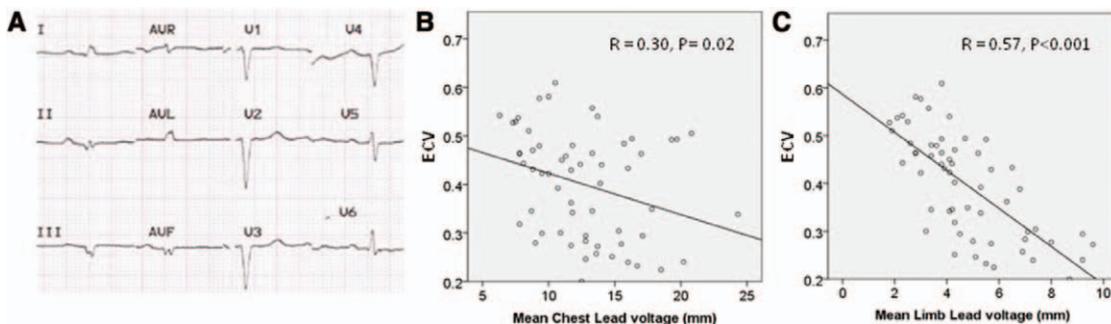


Figure 4. (A) Twelve-lead ECG showing low amplitude QRS voltages in the limb leads and pseudoinfarct pattern anteriorly. Extracellular volume fraction correlated only modestly with chest lead QRS voltages (B) but strongly with those in limb leads.

Although this series represents the largest prospective CMR study of the AL amyloid population, much further work is needed. As well as histological calibration, populations with ATTR amyloidosis merit study, as do the prognostic implications of ECV measurements and their reproducibility. Newer, single breath hold, T1 measurement techniques permit multislice mapping and have potential for whole heart quantification,^{21–23} holding the promise that it may be possible to avoid the gadolinium infusion in favor of a dynamic/pseudo equilibrium method, which would have substantial clinical advantages in terms of simplicity. Nevertheless, this preliminary study holds out promise for future new insights into cardiac amyloidosis and use of the EQ-CMR for being a reproducible method of serially quantifying the effects of existing and novel therapies.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Amyloid deposition within the extracellular space in the myocardium causes left ventricle thickening, but the extracellular space cannot currently be quantified accurately in amyloidosis. Here, the extracellular volume fraction is assessed using equilibrium contrast cardiovascular magnetic resonance and shows a number of correlations with current measures of left ventricle structure and function, as well as serum biomarkers and markers of functional capacity. We have shown that the extracellular volume derived from the equilibrium contrast cardiovascular magnetic resonance technique is clinically applicable, reproducible, and eliminates the effect of the altered gadolinium kinetics on the extracellular volume calculations. This has potential to be the first direct quantifier of myocardial amyloid and may allow monitoring of disease response after treatment with chemotherapy regimens.