

Extracellular volume with bolus-only technique in amyloidosis patients: diagnostic accuracy, correlation with other clinical cardiac measures and ability to track changes in amyloid load over time.

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ABSTRACT

Background: Extracellular volume (ECV) by T1 mapping requires the contrast agent distribution to be at equilibrium. This can be achieved either definitively with a primed contrast infusion (infusion ECV), or sufficiently with a delay post bolus (bolus-only ECV). For large ECV, the bolus-only approach measures higher than the infusion ECV, causing some uncertainty in diseases such as amyloidosis.

Purpose: To characterise the relationship between the bolus-only and current gold-standard infusion ECV in patients with amyloidosis.

Study Type: Bolus-only and infusion ECV were prospectively measured.

Population: 186 subjects with systemic amyloidosis attending our clinic and 23 subjects with systemic amyloidosis who were participating in an open-label, two-part, dose-escalation, phase 1 trial.

Field Strength: Avanto 1.5T, Siemens Medical Solutions, Ehrlangen, Germany

Assessment: Bolus-only and infusion ECV were measured in all subjects using ShMOLLI T1 mapping sequence.

Statistical Tests: Pearson correlation coefficient (r). Bland-Altman. Receiver Operating Characteristic (ROC) curve analysis. Linear regression model with a fractional polynomial transformation.

Results: The difference between the bolus-only and infusion myocardial ECV increased as the average of the two measures increased, with the bolus-ECV measuring higher. For an average ECV of 0.4, the difference was 0.013. The 95% limits of agreement for the two methods, after adjustment for the bias were ± 0.056 . However, cardiac diagnostic accuracy was comparable (bolus-only vs infusion ECV AUC=0.839 vs 0.836), as were correlations with other clinical cardiac measures, and, in the trial patients, the ability to track changes in the liver/spleen with therapy.

Conclusions: In amyloidosis, with large ECVs, the bolus-only technique reads higher than the infusion technique, but clinical performance by any measure is the same. Given the workflow advantages, these data suggest the bolus-only approach might be acceptable for amyloidosis, and might support its use as a surrogate endpoint in future clinical trials.

Keywords: Amyloidosis, magnetic resonance imaging, myocardial extracellular volume, bolus-only ECV, infusion ECV, trials.

INTRODUCTION

Systemic amyloidosis is a group of rare diseases characterized by amyloid deposition within the extracellular space[1]. Cardiac involvement is the main driver of prognosis [2], and is common in the two most prevalent forms of systemic amyloidosis, immunoglobulin light-chain amyloidosis (AL) and transthyretin amyloidosis (ATTR). The extent or severity of cardiac involvement in amyloidosis can be described by ventricular wall thickness, systo-diastolic dysfunction and the degree of transmural late gadolinium enhancement in magnetic resonance imaging (MRI) imaging [3], but none of these techniques provide a quantitative estimate of the amyloid deposits.

The burden of amyloid deposition can be quantified non-invasively with T1 mapping by cardiovascular magnetic resonance [2]. T1 mapping measures myocardial longitudinal magnetic relaxation, and can be performed before and after infusion of a gadolinium-based contrast agent. The contrast agent distributes to the extracellular space and blood plasma such that the relative contrast signal changes measure the myocardial extracellular volume fraction [4]. This measurement assumes that the distribution of the contrast agent within blood and myocardium is at equilibrium, established by the administration of a primed slow intravenous contrast infusion. However, this technique is complex and time-consuming.

An alternative approach involves approximation of the ECV utilizing the dynamic equilibration achieved by delayed post-bolus measurement [5]. This bolus-only technique has proven to be sufficient across a wide range of cardiac diseases[6], with advantages of speed and simplicity. However, there is an increasing bias between results of the bolus and infusion techniques in pathologies characterized by higher ECV, such as amyloidosis or the zone of a myocardial infarct [6]. Understanding the relationship between the two techniques for measuring ECV would be advantageous in order to apply the simpler bolus-only ECV

method to large clinical populations and imminent clinical trials of treatments created to eliminate amyloid deposits [7, 8].

The aims of the study were to: (1) characterise the relationship between the bolus-only and current gold-standard infusion cardiac ECV measurements in patients with amyloidosis and quantify the level of agreement between the two techniques; (2) compare the bolus and infusion ECV techniques in terms of: (a) cardiac diagnostic accuracy; (b) correlation with clinical cardiac measures; (c) reproducibility in the heart; and (d) ability to track changes in amyloid load in the liver and spleen in response to treatment.

METHODS

Bolus and infusion ECV measurements were performed in 2 distinct cohorts: 186 subjects with systemic amyloidosis (67 with AL and 119 ATTR amyloidosis) potentially involving the heart (cohort 1), and in 23 subjects with systemic amyloidosis (12 AL, 5 AFib, 3 ATTR, 2 AA and 1 with ApoA1 amyloidosis) variously involving the heart, liver and spleen who were participating in an open-label, dose-escalation, phase 1 trial that included repeated MRI assessments (cohort 2). Both cohorts were used to characterise the relationship between bolus and infusion cardiac ECV measurements. The first cohort was used to compare the two techniques in terms of cardiac diagnostic accuracy and correlation with other clinical cardiac measures. The second cohort was used to compare the two techniques in terms of reproducibility of the cardiac ECV measure and the ability to track changes in amyloid load in the liver and spleen in response to treatment.

Cohort 1. A total of 186 patients were classified into different groups: (1) 67 patients with biopsy proven systemic AL amyloid (40 male; aged 61 ± 11 years); (2) 119 with ATTR amyloidosis (95 male; aged 71 ± 11 years). Comparison of cardiac diagnostic accuracy, correlation with other clinical cardiac measures and quantification of level of agreement between bolus and infusion cardiac ECV have been assessed. Cardiac involvement was defined according to international consensus criteria as previously described [9] [10]. Patients with contraindication to contrast MRI examination, glomerular filtration rate <30 mL/min and/or MRI incompatible devices, were excluded from the study. The research received approval from UCL/UCLH Joint Committees on the Ethics of Human Research Committee. All participants provided written informed consent. Bolus and infusion cardiac ECV measures for all patients were included in the assessment of cardiac diagnostic accuracy, correlation with other clinical cardiac measures and the model to characterize the relationship between bolus and infusion cardiac ECV.

Cohort 2. A total of 23 systemic amyloidosis patients were enrolled in to an open-label, two-part, dose-escalation, phase 1 trial assessing the safety and pharmacodynamics of R-1-[6-[R-2-carboxy-pyrrolidin-1-yl][6]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid (CPHPC) co-administered with a fully humanized monoclonal IgG1 anti-serum amyloid P component (SAP) monoclonal antibody (12 AL, 5 AFib, 3 ATTR, 2 AA and 1 with ApoA1 amyloidosis; 12 male; age (mean \pm sd): 59 \pm 8 years) [7]. These patients were analysed by: comparison of reproducibility of bolus and infusion cardiac ECV; comparison of ability of bolus and infusion ECV to track changes in amyloid load in the liver and spleen in response to treatment; quantification of level of agreement between bolus and infusion cardiac ECV. MRI and SAP scintigraphy assessments were both performed at baseline and on day 42 and subjects had both bolus and infusion ECV measured in the heart, liver and spleen (ClinicalTrials.gov Identifier: NCT01777243).

All 23 subjects were included in the model to characterize the relationship between the bolus and infusion cardiac ECV measures. Subjects with both bolus and infusion ECV measures available and involvement in the organ of interest were included in the comparison of each techniques' ability to track changes in amyloid load (liver: n=9; spleen: n=13). Changes in liver and spleen were presented based on subjects' first and second pharmacologically-active dosing sessions respectively. Results from 22 subjects (out of 23) of the first dosing session in which subjects had both a baseline and day 42 cardiac ECV measure available were included in the assessment of reproducibility of both cardiac ECV measures (bolus-only: n=22; infusion: n=22). One subject was excluded from the assessment of reproducibility, as there was preliminary evidence of clearance of cardiac amyloid.

MRI protocol. All scans were performed on a 1.5T cardiac-enabled magnetic resonance imaging scanner (Avanto, Siemens Medical Solutions, Erlangen, Germany). Every patient received an initial contrast bolus of Gadoterate meglumine (gadolinium-DOTA,

marketed as Dotarem, Guerbet S.A., Paris, France) at a dose of 0.1 mmol/kg followed by measurement of the bolus only ECV (at 15 minutes), and a second contrast infusion of 0.1 mmol/kg started after a 15-min delay (immediately after post contrast T1 image acquisition) at 0.0011 mmol/kg/min for a minimum of 30 min. After a minimum of 30 min of infusion, ECV has been measured[6]. The Shortened Modified Look-Locker Inversion recovery (ShMOLLI) T1 mapping sequence was applied as previously described. Operators were allowed to perform standard cardiac planning with resulting variation in the following image acquisition parameters[11]: Number of Phase Encoding Steps = 105 ± 11 range = 74 to 143 median = 101 ms; TE = $0.5 * TR = 1.06 \pm 0.01$ range = 0.99 to 1.07 median = 1.07 ms; Percent phase field of view = 73 ± 8 , range = 51.04 to 98.96, median = 69.79; Acquisition matrix = 192 by 140 ± 15 , range = 98 to 190, median = 134; Phase partial Fourier 6/8; Slice thickness = 8 ± 0 , range = 5 to 8, median = 8 mm; Minimum TI = 103 ± 5 , range = 95 to 125, median = 100 ms, TI increment = 80 ± 0 ms. Imaging was performed with SSFP using flip 35° angle. Each image readout was preceded by 5 ramp up LISA pulses, and followed by a single 17.5° pulse at a TR/2 distance. Inversions were performed using a 10ms hyperbolic secant pulse.

MRI Analysis. A single region of interest (ROI) was drawn for each of the 4 required parameters for ECV as previously described (Figure 1): pre and post contrast T1 (myocardium, liver and spleen) and pre and post contrast blood T1 [12, 13]. All image analyses were performed blinded to the technique of ECV measurement. Hematocrit of all the patients was taken before each MRI study. The ECV was calculated with each method as:

$$ECV = (1 - \text{hematocrit}) \times \left(\frac{\Delta R1_{\text{myocardium/liver/spleen}}}{\Delta R1_{\text{blood}}} \right), \text{ where } R1 = 1/T1.$$

SAP scintigraphy acquisition and analysis. All patients in cohort 2 underwent whole body anterior and posterior scintigraphic imaging 24 h after administration of ^{123}I -labeled serum amyloid P component (SAP) using a GE Infinia Hawkeye gamma camera (GE

Healthcare, Iowa, MN) [14]. The labelled SAP studies were interpreted by a panel of physicians with experience of over 30,000 SAP scans. The amyloid load was categorised according to the intensity of ^{123}I -SAP uptake in the organs and the residual blood-pool signal at 24 hours after tracer injection. The categories of amyloid load were as follows: small (definite organ uptake but substantial blood-pool signal), moderate (more intense organ localization and reduced blood-pool signal), and large (very strong organ localization with little or no blood-pool activity) [14-20]. SAP scintigraphy was performed at baseline and on day 42. Changes from baseline in amyloid load were classified as better, stable or worse.

Statistical analysis. All analyses were performed post hoc using R version 3.2.3 (Vienna, Austria). The correlation between cardiac ECV and other clinical cardiac measures was assessed using the Pearson correlation coefficient (r). The level of agreement between the two techniques in measuring cardiac ECV and the repeatability of each technique was assessed using Bland-Altman methods[21]. A linear regression model with a fractional polynomial transformation was used to characterise the relationship between the difference and the average of the two measures of cardiac ECV and to quantify the 95% limits of agreement for the two methods [22].

Receiver Operating Characteristic (ROC) curve analysis was used to compare the diagnostic performance of the two techniques. Discrimination of cardiac involvement using bolus-only ECV and infusion ECV was quantified using the area under the ROC curve (AUC), where an AUC of 1 indicates perfect discrimination and an AUC of 0.5 indicates no better discrimination than that achievable by chance.

Box plots of subjects' bolus and infusion ECV measurements in the liver and spleen were presented by organ amyloid load (none, small, moderate, large) at baseline, as assessed by SAP scintigraphy. In addition, individual changes in bolus and infusion ECV measures in the

liver and spleen were presented by the change in amyloid load (reduced, stable) as assessed by SAP scintigraphy.

RESULTS

ECV comparison. There was a strong correlation between the cardiac ECV measures from the two techniques ($r^2=0.977$, 95% CI: 0.970 to 0.982) (Figure 2, left panel). The difference between the two cardiac ECV measures increased as the average of the two measures increased, with the bolus-only technique measuring higher (Figure 2, right panel). For an average ECV of 0.4 across the two measures, the difference was 0.013. This difference (i.e. bias) increased to 0.029, 0.054, 0.088, 0.134 for an average ECV of 0.5, 0.6, 0.7 and 0.8 respectively.

This relationship was best characterised using a linear regression with a cubic transformation on the average cardiac ECV. The 95% limits of agreement after adjustment for the bias were ± 0.056 .

Correlation with clinical cardiac measures and cardiac diagnostic accuracy. Bolus and infusion ECV showed similar correlation with relevant clinical cardiac measures (Table 1). The ability to identify patients with cardiac amyloidosis was similar for both techniques (bolus-only ECV: AUC=0.839, 95% CI=0.765 to 0.913; infusion ECV: AUC=0.836, 95% CI=0.760 to 0.912; n=185 (Figure 3).

Reproducibility. Better within-subject reproducibility was observed for the bolus cardiac ECV measure versus the infusion measure, with 95% repeatability bounds of 0.003 ± 0.036 (i.e. -0.033 to 0.039) and -0.006 ± 0.048 (i.e. -0.054 to 0.042) for bolus and infusion cardiac ECV respectively (Figure 4).

Tracking treatment response. Large amyloid loads as assessed by SAP scintigraphy were associated with an increasing trend in ECV measures in both the liver and spleen for both techniques, however this observation was made in a small sample of patients. Median bolus-only liver ECV measures were 0.350 (n=9), 0.395 (n=2) and 0.450 (n=8) for subjects with no,

moderate and large liver amyloid loads respectively. Similarly, the median bolus-only spleen ECV measures were 0.337 (n=4), 0.330 (n=7) and 0.575 (n=8) for subjects with small, moderate and large spleen amyloid loads respectively. Median infusion ECV values for both the liver and spleen were similarly greater for patients with larger amyloid loads (Figure 5). In patients with significant reduction in amyloid load in the liver (n=4) and spleen (n=4), as confirmed by SAP scintigraphy, comparable reductions were observed with the bolus and infusion ECV techniques, however this observation was made in a small sample of patients (Figure 6)

DISCUSSION

In cardiac amyloidosis, as expected [6], the bolus-only technique read higher than the infusion technique at high amyloid burdens. However, despite this bias both measures have comparable levels of discriminatory ability, correlate well with other clinical cardiac measures and offer the same ability to track reduction of amyloid in the liver and spleen with therapy. In addition, superior reproducibility was observed with bolus cardiac ECV compared with infusion cardiac ECV.

Our results support the implementation of cardiac ECV measurements in amyloidosis by the bolus-only technique in clinical practice. This has the advantage over the infusion technique of much shorter scan acquisition times, requiring a single breath-hold acquisition before and ~15 minutes after the contrast agent administration (or a total of 6 breath-holds to cover a “whole-heart” approach). We have also demonstrated the feasibility of the technique in ECV measurements in the spleen and the liver, with an average increase in the acquisition time of only approximately 3 minutes. Advances in the post-processing component of ECV measurement, such as extracellular volume maps, may also facilitate the clinical utility of the technique. ECV maps (with and without the use of hematocrit measurement, the latter known as “synthetic ECV” maps [23]) are now available, offering a rapid automated approach to generate online pixel-wise ECV maps, enabling the clinician to obtain ECV values immediately after the acquisition of post-contrast T1 maps [24].

The findings from this study might also extend beyond clinical utility. The development of therapies to reduce production of amyloid or enhance its clearance has gained substantial momentum [7, 8, 25, 26]. Several molecules are being developed that are able to specifically suppress amyloid production or enhance the clearance of amyloid deposits. However, the lack of available methods to track changes in amyloid load over time has been a major problem for drug development. Infusion and bolus ECV have been used as

exploratory endpoints in an open-label, two-part, dose-escalation, phase 1 trial assessing the safety and pharmacodynamics of CPHPC (a SAP depleter) followed by an anti-SAP monoclonal antibody [7]. In patients in whom major clearance of non-cardiac amyloid was demonstrated with the use of other methods, both infusion and bolus-only ECV confirmed that extracellular volume in the organ of interest decreased towards normal. Whilst the use of infusion ECV, although cumbersome, is feasible in small, phase 1 single centre trials, the implementation of the infusion technique in larger multicentre trials could be extremely challenging, highlighting the importance of these study findings.

This study has some limitations. Whilst our results demonstrate the differences between the bolus and infusion ECV techniques, they do not provide the underlying reasons to account for them. The bolus-only technique assumes that a dynamic equilibrium occurs with sufficient delay post-bolus (15 minutes), because blood/myocardial exchange rate constants are much faster than other effects that influence blood gadolinium concentration (such as renal excretion). For amyloid, however, the increasing difference between the bolus and infusion techniques for increasing cardiac ECV values points towards additional processes potentially influencing the contrast kinetics, such as interaction between the contrast agents and amyloid deposits. A histological approach with cardiac biopsies has been used to validate the bolus-only technique in diffuse fibrosis. However, the patchy nature of amyloid deposition and hazards of biopsy pose challenges to this approach in cardiac amyloidosis, and the basis for the increasing difference between the two techniques for increasing ECV states in amyloid remains unknown. Furthermore, the study has been conducted in only one scanner, with no available data on the reproducibility between different scanners or reproducibility using different parameters. Although no data are available for ECV in the literature, native myocardial T1 has been proven to be a robust and reproducible biological parameters in large multicenter studies[11].

In conclusion, we have demonstrated the bolus-only ECV technique to be feasible and accurate when applied to a large patient population with cardiac amyloidosis. After accounting for the known bias of the bolus-only technique for increased ECV measurements at higher ECV levels, there remained a good level of agreement with the traditional infusion technique. This allows a more rapid protocol for scan acquisition that can be applied to both clinical practice and to the development of multi-centre clinical trials in cardiac amyloidosis.

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Table 1: Correlation between bolus and infusion ECV with MRI and echocardiography metrics of cardiac function, 6 minute walk test, cardiac biomarkers and ECG.

Characteristic	N (%)	Median (IQR: 25 th to 75 th percentile)	Pearson correlation (95%CI)	
			ECV by infusion	ECV by bolus only
<i>LV Structure (MRI)</i>				
LV mass _i (g/m ²)	182 (98%)	107.86 (80.16, 137.64)	0.67 (0.58, 0.74)	0.67 (0.58, 0.74)
LA area _i (cm/m ²)	179 (96%)	15.00 (12.43, 17.43)	0.45 (0.33, 0.56)	0.46 (0.34, 0.57)
SV _i (mL/ m2)	182 (98%)	40.84 (32.62, 46.42)	-0.38 (-0.50, -0.25)	-0.42 (-0.53, -0.29)
LVEF (%)	183 (98%)	62.00 (50.00, 70.92)	-0.55 (-0.65, -0.44)	-0.61 (-0.69, -0.50)
<i>LV diastolic function (Echocardiogram)</i>				
E/E	183 (98%)	13.71 (8.82, 19.25)	0.34 (0.20, 0.46)	0.32 (0.18, 0.44)
E-deceleration time (msec)	185 (99%)	178.00 (146.00, 215.00)	-0.24 (-0.37, -0.01)	-0.22 (-0.35, -0.08)
6-minute walking distance (m)	131 (70%)	304.00 (220.50, 420.50)	0.00 (-0.17, 0.17)	0.02 (-0.15, 0.19)
<i>Biomarkers</i>				
NT-proBNP(pmL/L)*	186 (100%)	210.00 (66.00, 441.50)	0.73 (0.652, 0.79)	0.70 (0.62, 0.77)
Troponin T (pmol/L)*	124 (67%)	0.06 (0.03, 0.08)	0.63 (0.513, 0.73)	0.60 (0.48, 0.70)
<i>ECG</i>				
PR (msec)	113 (61%)	182.00 (154.00, 212.00)	0.43 (0.27, 0.57)	0.45 (0.29, 0.58)
QRS (msec)	168 (90%)	98.00 (90.00, 120.00)	0.33 (0.18, 0.45)	0.35 (0.21, 0.47)
ECG limb lead mean voltage	173 (93%)	4.33 (3.33, 6.50)	-0.31 (-0.44, -0.17)	-0.31 (-0.44, -0.17)

Note: ECG = electrocardiography, LA area = left atrial area indexed, LV_i mass = left ventricle mass indexed, LVEF= left ventricle ejection fraction, SV_i = stroke volume indexed.

* Correlation coefficient and 95% CIs calculated on log_e transformed data.

Figure 1. Examples of ROIs in ShMOLLI. 4-chamber: ShMOLLI (A) pre-contrast and (B) post-contrast with ROIs drawn in the blood and in the basal septum.

Figure 2. Correlation and agreement of bolus and infusion cardiac ECV measures: scatter plot of bolus vs infusion cardiac ECV (Panel A) and Bland-Altman comparison of bolus and infusion cardiac ECV measures (Panel B).

Pearson correlation coefficient (r) = 0.977 (left panel)

Linear regression with cubic transformation on average ECV:

- intercept = -0.005 (95% CI: -0.011 to 0.002)
- slope = 0.271 (95% CI: 0.236 to 0.306)
- 95% limits of agreement (after bias adjustment) = ± 0.056
- E.g. with an average ECV of 0.50, the difference (i.e. “ECV bolus-only” minus “ECV infusion”) is estimated as $-0.005 + 0.271 \cdot (0.50^3)$ which is equal to 0.029

Figure 3. Receiver operating characteristic (ROC) curve for the discrimination of cardiac amyloidosis by ECV with bolus-only technique (left panel) and ECV with infusion technique (right panel).

Figure 4. Repeatability of bolus-only and infusion cardiac ECV measures. Scatter-plot of baseline versus day 42 follow-up bolus-only ECV (A) and infusion ECV (B). Bland-Altman comparison of two repeat bolus (C) and infusion (D) ECV measures.

Figure 5. Association between ECV at baseline (infusion and bolus-only techniques) and baseline amyloid load as assessed by SAP scintigraphy in the liver (A) and the spleen (B).

Figure 6. – Changes in liver ECV (infusion and bolus-only techniques) vs changes in liver amyloid load as assessed by SAP scintigraphy (Left panel); changes in spleen ECV (infusion and bolus-only techniques) vs changes in spleen amyloid load as assessed by SAP scintigraphy (Right panel).