Longitudinal Strain is an independent predictor of survival and response in patients with systemic AL amyloidosis – analysis of 915 patients from ALCHEMY prospective study

Oliver C Cohen[1], Andreia Ismail[1], Richa Manwani[1], Sriram Ravichandran[1], Steven Law[1], Darren Foard[1], Sevda Ward[1], Babita Pawarova[1], Brooke Douglas[1], Ana Martinez De Azcona Naharro[1], Shameem Mahmood[1], Sajitha Sachchithanantham[1], Helen J. Lachmann[1], Philip N. Hawkins[1] Julian D. Gillmore[1], Marianna Fontana[1], Rodney H Falk[2], Carol J. Whelan[1] and Ashutosh D. Wechalekar[1]

[1]National Amyloidosis Centre, University College London, London, United Kingdom.
[2]Brigham and Women's Hospital, 75 Francis Street, Boston, MA, USA.

Total word count of manuscript inclusive of title page, abstract, text, references, tables and figure legends: **7236**

Corresponding author:

Professor Ashutosh Wechalekar
National Amyloidosis Centre
UCL (Royal Free Campus),
Rowland Hill Street
London, United Kingdom
Email: a.wechalekar@ucl.ac.uk
Phone: +440207 433 2816

Abstract

Background: Cardiac involvement, a major determinant of prognosis in AL amyloidosis, is characterised by an impairment of longitudinal strain (LS%). We report the utility of LS% in an unselected series of patients with systemic AL amyloidosis.

Methods: 915 serial newly diagnosed AL patients with comprehensive assessments at baseline, 12 and 24 months were included.

Results: 628/915 (68.6%) patients had cardiac involvement. The baseline LS% worsened with increasing Mayo stage. Stages I, II, IIIa, IIIb had median LS% of -21.2%, -17.5%, -12.0% and -9.0% respectively ($p<0.0001$). Baseline LS% stratified patients in three cohorts (LS% ≤-17%, -16.9- -10.3% and ≥-10.2%). Overall survival by cohort was: ≤-17%: not reached; -16.9% - -10.3%: 36 (95% confidence interval [CI] 24.9-47.1) months and ≥-10.2%: 7 (95% CI: 4.5-9.5) months with each cohort being an independent predictor of survival ($p<0.0001$). Improvement in LS% was seen from 12 months onwards in patients achieving a complete haematologic response (CR) (median improvement from -13.8% to -14.9% at 12 months in those with CR and deep free light chains response). Patients with any improvement in strain had better overall survival (at 24 months: median 72 months vs. not reached, $p=0.007$). Patients achieving a LS% improvement survived longer than those achieving a standard biomarker cardiac response ($p<0.0001$).

Conclusion: Baseline LS% is a functional marker that correlates with worsening cardiac involvement and poorer LS% and was an independent predictor of survival. Improvement in LS% was seen in patients with a complete haematologic response and was associated with significantly better long-term survival. Baseline LS% and an absolute improvement in LS% should be considered as a criterion for prognosis and response in cardiac AL amyloidosis, respectively.

Clinical Perspectives

What is new?

- Longitudinal strain is an independent predictor of survival in patients with cardiac AL amyloidosis at baseline and can stratify risk based on worsening strain measurements
- Longitudinal strain improves after treatment but only in patients in a complete haematological response (especially in those with a low final dFLC of <10mg/L)
- An improvement in longitudinal strain of -1.5% or more translates to improved survival

What are the clinical implications?

- Baseline longitudinal strain should be considered as a prognostic criterion at baseline
- Serial strain measurement is an additional marker to assess cardiac response after chemotherapy and lack of improvement/worsening suggests need for deeper suppression underlying clonal disease
- An absolute improvement in longitudinal strain of -1.5% defines a new group with a better prognosis and can be a potential end for clinical trials in AL amyloidosis

Introduction

Systemic AL amyloidosis is characterised by the misfolding of light-chain immunoglobulin, produced by a clonal plasma cell population in the bone marrow, and its deposition in organs leading to progressive tissue damage (1). Cardiac involvement (seen in approximately three-quarters of cases) is the major determinant of prognosis (2). Diagnosis of cardiac involvement in amyloidosis relies on imaging and biomarkers in the appropriate context. Cardiac magnetic resonance imaging (CMR) is highly sensitive for amyloid diagnosis (3) but not widely available and cannot be used in patients with devices or significant renal impairment. Echocardiography is widely available and is the usual first-line investigation. Cardiac biomarkers, N-terminal probrain natriuretic peptide (NT-proBNP) and troponin form prognostic staging systems (originally developed by the Mayo group – stage I-III) (4) further refined by our European collaborative group showing that NT-proBNP >8500ng/L independently predicted overall survival (OS) and defined a new sub-group within the original Mayo stage III with a particularly poor prognosis of just 3 months (now termed as Mayo IIIb) (5).

Even in the setting of biopsy-proven amyloid in another organ, a defining criterion for cardiac involvement of increased left ventricular (LV) wall thickness of >12mm in the absence of another cardiac cause remains rather non-discriminatory (6). The early loss of longitudinal cardiac function detected by use of longitudinal strain (LS%) has emerged as a sensitive marker of cardiac amyloidosis with relative apical sparing of LS impairment amongst the most specific findings (7). . LS% also appears to be a predictor of survival (8-13). Stroke volume index (SVI) (12) , myocardial contraction fraction (MCF) (12), E/e', LV mass (14) and longitudinal early diastolic strain rate (15) appear to predict survival but are not widely used. Large studies of LS% in a uniformly treated AL population remain lacking.

Monitoring for cardiac improvement (or worsening) relies on NT-proBNP as a marker reflective of mechanical stress on cardiac myocytes by amyloid deposits as well as direct proteotoxicity of the amyloidogenic light chains/oligomers (16) but its remarkable sensitivity to

changes in fluid balance can lead to challenges. Change in LV wall thickness by echocardiogram is poorly reproducible and rarely seen. Improvement in longitudinal strain has been reported in a retrospective study of 61 patients and correlated with NT-proBNP response (9). Early improvement in LS% has been observed following administration of the monoclonal anti-fibril antibody CAEL-101 in a phase 1 study of 19 patients (17).

This a first report of a large cohort of uniformly treated prospectively followed patients with AL amyloidosis assessing the impact of baseline 2D longitudinal strain on survival, assessment of changes to LS% following treatment and their impact on outcomes.

Method

Patient Population

All patients from a prospective observational study of newly diagnosed AL amyloidosis (ALCHEMY) seen at the UK National Amyloidosis Centre (NAC) (February 2010 – August 2017) were included within the study. All patients received bortezomib-based treatment first-line. The diagnosis of AL amyloidosis was confirmed by central review of histological material inclusive of Congo red staining. Amyloid subtype was identified by immunohistochemistry with specific antibodies, or by mass spectrometry. All patients underwent a comprehensive baseline assessment inclusive of blood monitoring of organ function and clonal parameters and echocardiography. Subsequent assessment occurred at 12 and 24 months at the NAC.

Amyloidotic organ involvement, haematological responses to chemotherapy and organ responses were defined as per consensus criteria published by the international society of amyloidosis (6, 18, 19). Cardiac involvement is defined by a mean LV wall thickness of >12mm on echocardiogram in the absence of an alternative cause. Patients who underwent cardiac magnetic resonance imaging (CMR) and were confirmed to have features characteristic of cardiac amyloidosis were also adjudged to have cardiac involvement irrespective of LV wall thickness. Cardiac staging

was defined by the European modification of the Mayo 2004 staging to stratify patients as: Mayo stage I, Mayo Stage II whilst Mayo stage III patients were subdivided into IIIa (NT-proBNP <8500ng/L) and IIIb (NT-proBNP ≥8500ng/L) (5). A cardiac organ response is defined by reduction in NT-proBNP (>30% and >300ng/L) (only assessable in patients with baseline NT-proBNP ≥650ng/L) or ≥2 class decrease in the New York Heart Association (NYHA) class (only assessable in patients with a baseline class of 3 or 4). Cardiac progression is defined by an increase in NT-proBNP by both >30% and >300ng/L, ≥33% increase in troponin or a ≥10% decrease in LV ejection fraction. Haematological CR is defined by the absence of a detectable monoclonal protein in serum or urine with normalisation of the free light chain (FLC) ratio. A very good partial response (VGPR) represents a difference between involved and uninvolved light chains (dFLC) of <40mg/L (in the absence of a CR) whilst a partial response (PR) represents a dFLC decrease of >50% from baseline. Finally, given recent reports of the benefits of a deep reduction in dFLC on outcomes (20), we assessed absolute dFLC terming a dFLC <10mg/L as a ''stringent dFLC response'' (as previously published(20)). Overall survival was calculated from the date of diagnosis of amyloidosis to death from any cause.

Echocardiography

All echocardiograms were performed using a GE Vivid E9 ultrasound machine equipped with a 5S probe and measurements performed offline using Echo PAC software (Version 202). The overall, basal and apical LS%, LV ejection fraction and LV wall thickness were performed and calculated in accordance with previously published guidance (21). Specifically, all strain and strain-derived variables were measured in the apical 4-chamber view. Peak longitudinal strain (LS) was computed automatically, generating regional data from 6 segments (basal, mid, apical interventricular septum and basal, mid, apical lateral wall), to calculate an average value. Strain-derived variables were acquired and calculated according to previous studies: septal longitudinal systolic apex to base (SAB) ratio (22) and relative apical longitudinal strain (RALS) as the average 4-chamber apical segments peak longitudinal strain/average basal and mid 4-chamber peak longitudinal strain.(23)

Echocardiograms were analysed by an experienced echo-cardiographer and were re-analysed by a second experienced operator to ensure standardisation of measurements. In order to ensure that these measurements were reproducible, the strain measurements from a serial sample of 10% of the baseline echocardiograms from patients with cardiac involvement by AL amyloidosis were analysed by a second experienced operator blinded to any prior strain measurements with a very high positive correlation. (Pearson correlation; r=0.937 [95% CI: 0.897-0.961], n=62, p<0.0001).

Statistical Analysis

Statistical analysis was performed using SPSS version 25. Approval for analysis and publication was obtained from the National Health Service institutional review board; written consent was obtained from all patients in accordance with the Declaration of Helsinki. The Kaplan-Meier method was used to analyze survival outcomes. Multivariate modelling by Cox regression analysis was performed to assess the impact of Mayo stage and LS% on survival. Two-tailed unpaired t-tests were used to compare continuous variables whilst analysis of variance (ANOVA) was used when >2 variables were included. All p-values were 2-sided with a significance level of <0.05. Receiver operating characteristic (ROC) curves were plotted to determine the optimal sensitivity and specificity of longitudinal strain for survival. Pearson correlation was used to assess inter-observer variability in measuring LS%.

Results

The impact of LS% at baseline

In total, 915 patients were included in the study of which 628 (68.6%) patients had cardiac involvement including 25 patients whose LV wall thickness did not meet consensus criteria for cardiac involvement (i.e. LV wall thickness not >12mm) but in whom CMR showed characteristic features of amyloidosis. Baseline cardiac staging was - Stage I: 144 (15.7%), Stage II: 302 (33.0%), Stage IIIa: 344 (37.6%) and Stage IIIb: 125 (13.7%). Further baseline characteristics are included in

Table 1. A consort diagram (Figure 1) shows the numbers of patients with evaluable echocardiograms at each time point. There were significant differences at baseline between those with and without cardiac involvement (Supplementary Table 1 and 2). The median baseline LS% was -15.2% for the entire cohort and was significantly poorer in those with cardiac involvement -12.1% (vs. -21.1% without cardiac involvement; p<0.0001). The LS% worsened with advancing cardiac stage with median -21.2%, -17.5%, -12.0% and -9.0% for stage I, II, IIIa , IIIb respectively (p<0.0001) (Figure 2). In patients with cardiac involvement who died within 6 months of diagnosis, median LS% was -9.3% (n=188) compared to -13.6% (n=428) in those who survived >6 months (p<0.0001).

The median OS of the entire cohort was 61 (95% CI: 49.9-72.1) months (Figure 3A) and was 31 (95% CI: 23.9-38.1) months in patients with cardiac involvement. Median OS for Mayo stages I and II was not reached whilst stages IIIa and IIIb were 30.0 (95% CI: 23.1-36.9) and 4.0 (95% CI: 1.8-6.2) months respectively. We stratified patients based on presenting LS%. A ROC analysis was used to determine a high LS% cut-off in patients with cardiac involvement to discriminate survivors from non-survivors for use in regression analysis. A cut off of -17% was selected (89% sensitivity, 68% specificity). Salinaro and colleagues (9) reported a ≥-10.2% cut-off associated with a 25.1% likelihood of survival at 12 months and was close to our median for early deaths (LS%: -9.3) within our cohort discriminating patients with a particularly poor prognosis. We have chosen to use the published -10.2% as the lower threshold. Consequently, we stratified patients with cardiac involvement into three distinct categories: those with a baseline LS% ≤-17.0%, between -16.9 - -10.3% and ≥-10.2%. There was highly significant worsening OS with worsening LS% categories: LS% ≤-17.0% - OS was not reached, LS% between -16.9 - -10.3% - median OS 36.0 (95% CI: 24.9-47.1) months and LS% ≥-10.2% - median OS 7.0 (95% CI: 4.5-9.5) months (p<0.0001) (Figure 3B). There was overlap between the LS% categories and Mayo cardiac stage (LS% ≥-10.2%: Stage II: 12.8%, Stage IIIa: 55.7% and Stage IIIb: 31.5%; LS% -16.9 - -10.3%: Stage I: 1.6%, Stage II: 34.6%, Stage IIIa: 47.9% and Stage IIIb: 16.0% and LS% ≤-17%: Stage I: 2.5%, Stage II: 55.0%, Stage IIIa: 39.2% and Stage IIIb: 3.3%).

On univariate analysis, baseline LV wall thickness, LV ejection fraction and both overall and regional LS% parameters were predictive of survival (Supplementary Table 3) as were the cardiac biomarkers NT-proBNP (Hazard ratio 2.58 [95% CI 2.09-3.19], p<0.0001) and Troponin T (Hazard ratio 3.61 [95% CI 2.56-5.08], p<0.0001). On multivariate analysis including Mayo cardiac staging, the LS% cut-offs (≤-17%, -16.9 - -10.3%, ≥-10.2%) remained independent predictors of survival (Table 2).

The impact of a change in LS% on survival

We assessed serial strain at baseline and 12 months in 342 patients evaluable at both time points (225 died before 12 months and 49 missing data or uninterpretable strain). We also assessed this at 24 months (n=236). Patients with any improvement in strain had better OS (72.0 [95% CI: 57.0-87.0] months vs. not reached, p=0.007). However, to make this more reliable, we deemed that a minimum improvement in overall LS% of -1.5% would be more reproducible and clinically meaningful than simply 'any improvement' and termed a -1.5% improvement as "LS-Response". Among patients who had at least -1.5% improvement in LS% at 12 months, subsequent median OS was not reached, compared to a median survival of 72.0 [95% CI: 65.1-78.9] months among those not achieving a clinically meaningful strain improvement, p=0.008). An improvement of -1.5% by 24 months was also significant (80.0 [95% CI: 74.6-90.5] months vs. not reached, p<0.0001) (Figure 3C). An improvement in LS% of -1.0% and -2.0% were also highly significant at both 12 (p<0.0001 and p=0.001 respectively) and 24 months (p<0.0001 and p=0.001 respectively). Patients who did not achieve a -1.5% improvement at 12 months but had improved by -1.5% at 24 months had not reached median OS (vs. 80.0 months in those who hadn't achieved a -1.5% improvement at either 12 or 24 months but this was not statistically significant (p=0.06).

The impact of haematological and cardiac organ response on LS%

Changes in LS% were evaluated according to depth of haematological response. In total, 325 patients with cardiac involvement had evaluable echocardiographic parameters at both baseline and 12 months from commencement of treatment (thus deaths prior to 12 months are excluded) and

were evaluable for haematological responses. The haematological responses were: CR – 82 (25.2%), VGPR – 143 (44.0%), PR – 71 (21.8%) and NR – 29 (8.9%). Improvements in LS% were seen in patients achieving a CR and LS% mostly worsened in patients with less than a complete haematological response at 12 months. At the earlier time point of 6 months none of the groups showed any improvement in LS% (including those who reached a CR) although those who failed to achieve any haematologic response had significant worsening (-14.6% to -12.4%, p=0.0005) (Supplementary Table 3).

In patients achieving a CR, both overall and basal LS% improved (p=0.04 and p=0.007 respectively) (Table 3). Of patients in CR, 32/82 (39.0%), achieved a LS-response (≥-1.5% improvement) at 12 months. Patients achieving a LS-response and complete haematological response survived longer than those achieving a CR alone (not reached vs. 80 months, p=0.004). At 24 months, 66 of these 82 patients in CR (80.5%) were evaluable (10 missing data, 2 died, 2 not reached 24 months, 1 not assessable due to presence of LV-assist device) with 40 (60.6%) achieving a LS-response. Patients who consistently remained in a CR up to 24 months (n=42) had an improvement in LS% from a median of -13.5% at baseline to -15.6% at 24 months (p=0.0002) with 66.7% achieving a LS-response. For patients whose underlying disease progressed from an initial CR prior to the 24 month assessment, only 52.2% patients had a LS% response (p=0.0004).

Since free light chains are the drivers of disease in AL amyloidosis, we assessed the impact of deeper light chain response on LS% improvement. For patients reaching very low dFLC after treatment - dFLC<10mg/L (n=132), demonstrated a median improvement in LS% of -0.8% (p=0.01) compared to a +0.8% worsening in those achieving a dFLC response of 10-40mg/L at 12 months (p=0.003). Seventy two patients met criteria for both dFLC<10mg/L and a complete haematological response demonstrating a median -1.1% improvement in LS% (p=0.02). On the contrary, patients meeting criteria for a complete haematological response but continued to have a dFLC >10mg/L (n=60) did not demonstrate a significant improvement in LS% at 12 months (p=0.32) (Table 4).

Conversely, in patients who did not achieve a CR, there was worsening of echocardiographic parameters, which was most marked in non-responders (median reduction in LS% from -14.6% to -12.6%) (Table 2).

We additionally assessed the changes in LS% in relation to cardiac organ response as defined by cardiac biomarkers. There were 203 patients assessable for both a cardiac organ response and changes in LS% at baseline, 12 and 24 months (Non assessable: 21 baseline NT-proBNP <650ng/L, 11 missing data, 1 dialysis). Of evaluable patients (n=203), 133 (65.5%) achieved a cardiac organ response by biomarkers, rising to 80.3% (49/61) in those achieving a haematological CR. A further 5 patients who had achieved CR at 6 months died prior to the 12 month assessment. Patients achieving a cardiac organ response demonstrated highly significant improvement in both overall (-12.8% to -14.4%, p<0.0001) and basal LS% (baso-lateral: -7.9% to -10.1%, p=0.0002; baso-septal: -6.1% to -7.6%, p<0.0001) whilst LS% deteriorated (-14.4% to -13.4%, p=0.004) in the patients who did not have cardiac biomarker organ response (Table 5).

Patients who achieved a cardiac response at both 12 and 24 months survived longer (p<0.0001). Surviving patients who failed to achieve a cardiac response at 24-month assessment had a median OS of 72.0 (95% CI: 50.5-93.5) months vs. not reached in those achieving a cardiac response. Patients who met criteria for both a LS-response and a cardiac response lived longer than those achieving a cardiac response alone although median survival was not reached in either case (p<0.0001) (Figure 3D).

Discussion

This study confirms the importance of LS% in the assessment of cardiac AL amyloidosis at baseline and, crucially, for serial monitoring to assess cardiac improvement. Baseline LS% is an independent predictor of overall survival (over and above the standard biomarker-based criteria). LS% improved after successful treatment of AL amyloidosis. However, this study crucially shows that LS% only improves in patients who achieve a haematological CR. A clinically meaningful LS%

improvement of -1.5% at 12 and 24 months after initiation of chemotherapy improves subsequent long-term survival.

The goals of treatment in systemic AL amyloidosis are to eventually achieve improvement in the function of organs affected by amyloidotic deposits. However, as yet, there are no drugs that can directly achieve this and the treatment is focused on rapid profound reduction of the amyloidogenic free light chains to curtail amyloid deposition within organs and prevent consequent tissue damage, allowing for natural macrophage-led amyloid clearance (18). Cardiac involvement remains the main determinant of prognosis. Biomarker based staging systems, the Mayo classification (and its European modification) as well as an updated version incorporating FLCs, has robustly stood the test of time as crucial prognostic systems in AL amyloidosis. Imaging for amyloid deposits has not been widely adopted for prognosis and has, almost, never been shown to be independent of the Mayo defined criteria. Over the years various echocardiographic parameters including wall thickness, E/E', MAPSE have been reported for prognosis. The amyloid load in the heart as determined by interstitial volume fraction by CMR as well as myocardial oedema determined by T2 weighted imaging appear to have a role (24).

In this study, we show that LS% is an independent marker of outcomes for patients with AL amyloidosis with cardiac involvement in an unselected group of uniformly treated patients. Previously, LS% ≤-17% was shown to discriminate survivors and non-survivors in 82 patients following autologous stem cell transplant (ASCT) (11). Another study with 238 AL patients showed that LS% worse than -14% was associated with worse outcomes (12). A third study of 61 patients identified a lower cut off of -10.2% (9) to discriminate patients with a particularly poor prognosis. It seems clear from these studies as well as the current cohort, that poorer LS% is associated with worse outcomes (in our series the median LS% of patients who died within six months was -9.3%). One of the limitations of other studies is inclusion of patients without cardiac involvement in the models – by definition, they will have better LS% and prognosis. Hence, we deemed it critical that

the determination of any cut-off included only patients with cardiac involvement so that the defined value is not impacted by any patients without cardiac involvement. We assessed the impact of various cut offs – both higher and lower. We show clearly that when stratified by baseline LS% (≤-17%, -16.9- -10.3% and ≥-10.2%), there are three clear prognostic groups with overall survival significantly better in the higher LS% category. We found that our LS% cut offs provided incremental survival data (Table 2) above that provided by current Mayo stage criteria including the very poor prognostic stage IIIb group. There is almost no other variable that has been previously useful to show independent prognostic information over that provided by a very high presenting baseline NT-proBNP >8500 ng/L. These data show that baseline LS% provides an additional robust marker for baseline prognosis and, if validated in larger international collaborative studies, should be incorporated in a new updated prognostic staging for patients with cardiac AL amyloidosis.

Monitoring of treatment response in AL amyloidosis is twofold – the haematologic response using serological markers as described and change in amyloidotic organ function. The former is now well established but accurate assessment of change in organ function, especially cardiac function, which is crucially required for treatment decisions has remained sub-optimal. Current algorithms advocate further plasma cell-directed therapy in patients who fail to achieve a very good partial response (VGPR, dFLC <40mg/L) with either systemic chemotherapy or ASCT (25, 26). Since chemotherapy or ASCT remain the standard of care for patients with AL amyloidosis, treatment toxicity is considerable and avoidance of both over or under treatment is important. For assessing cardiac response, NT-proBNP remains an important marker. Thus far, objective easily accessible functional assessments have remained elusive. The LS% data from this study shows critical novel observations that may offer LS% (and changes after treatment) as one such marker. At six months, patients do not show functional cardiac improvement by LS% even if they achieve a good but not a complete clonal response. These observations are in keeping with long known fact that patients with cardiac amyloidosis continue to die even after they achieve a chemotherapy response, due to heart failure or sudden cardiac death, and the reason is likely to be lack of cardiac response.

We attempted to define a minimum improvement in LS% that may provide clinically meaningful and easily reproducible data but there was limited information to guide this for amyloidosis. Prior studies have documented the value of LS% recovery (e.g. post myocardial infarct (27)) but there is no published data defining a degree of LS% improvement that is considered clinically meaningful. Any attempt to define such a value must account for both the inter-observer variability in calculating LS% and the impact of such a change on function. In addition, LS% measurements may vary slightly with the imaging software used to calculate it (10) and the impact of these differences, while small needs to be further elucidated. We found that LS% calculated by a second independent observer correlated well with initial LS% measurements ($r=0.937$, $p<0.0001$), indicating the robust nature of this measurement. The current cohort clearly shows that any improvement conferred a survival advantage, but we adjudged that -1.5% likely constituted the minimum value that would lead to clinically meaningful and easily reproducible criteria. Cardiac response is traditionally defined by a 30% NT-proBNP improvement and 300ng/L decrease (assuming baseline ≥650ng/L) whilst progression is determined by deterioration in NT-proBNP, cardiac Troponin, NHYA class and ejection fraction (19) Patients achieving a -1.5% LS% improvement had significantly better OS and we have term this as "LS-response". In complete hematologic responders, there was a significant improvement in overall and regional LS% with a median improvement of -1.6% by 24 months, a clinically significant change. Conversely, overall LS% worsened, together with LV wall thickness and LVEF in haematologic non-responders. A LS% response of -1.5% provided incremental information to further stratify patients who had achieved cardiac response by international consensus criteria, defining a separate group with an improved prognosis. These data support that an absolute improvement in LS% should be considered as an additional criterion for cardiac response in AL amyloidosis and, hence, needs validation by the International Society of Amyloidosis in an international collaborative series.

Improvement in LS% (overall and basal) was seen in patients in a haematologic CR, defined by the absence of a monoclonal protein detectable in serum or urine in combination with a normal

FLC ratio (19). Previously, Salinaro and colleagues (9), had demonstrated a trend towards significant improvement in basal strain in 18 CR patients (p=0.13). The finding that basal (septal and lateral) LS% improved most significantly reflects relative apical sparing by amyloid and, utilising greater patient numbers, corroborates and extends findings of an improved apical to basal strain ratio in patients in CR (9). However, even in patients in a CR, there was no significant improvement at 6 months highlighting the limitations of current therapies that do not affect the fibril deposits directly. Whilst the median changes seen in patients achieving CR at 12 months were not great enough to be considered clinically meaningful (i.e. not meeting the -1.5% improvement threshold), they reinforce the notion that a degree of improvement in echocardiographic markers is unique to a CR. By 24 months, two-thirds of patients in a persistent CR had achieved a LS-response of -1.5% or greater and crucially a substantially greater proportion of patients who maintained the CR had this improvement than patient who progressed before 24 months showing the need to maintain an ongoing response for a long time for true functional recovery.

Since light chains are the driver of disease in AL amyloidosis, we assessed the impact of deep reduction in FLCs with patients who had or did not have complete elimination of the intact monoclonal protein component (i.e. with and without a "CR"); using a previously defined dFLC threshold of dFLC <10mg/L (28). Patients achieving a dFLC<10 mg/L show improvements in LS% whereas those achieving a CR without a dFLC <10mg/L do not – a crucially important observation for guiding therapy. Our group have previously reported that this stringent dFLC response translates to significantly longer time to next treatment and two-thirds of patients achieving cardiac responses thus potentially defining a new goal of therapy in AL amyloidosis (20). Now, we demonstrate an improvement in strain in this subgroup that is not seen in patients in CR who fail to achieve a dFLC<10mg/L reinforcing its importance as a potential goal of therapy.

This study is limited by its single centre design and the fact that there some data is missing at each time point reflecting patients either lost to follow up or choosing not to return to the NAC for

follow up. Given the nature of the NAC as a national referrals centre, patients unable to travel to the NAC at first diagnosis and thus representing a more unwell cohort may be underrepresented in our analysis. In some sub-analyses of the study, patient numbers are small and results need further confirmation. Whilst we assessed inter-observer variability within our centre, this needs to be assessed across different centres for reproducibility.

In summary, baseline LS% is predictive of survival and independent of the standard biomarker-based system for defining prognosis in patients with AL amyloidosis with cardiac involvement. LS% improves after treatment but improvements are slow. Only patients with a complete haematologic response (and especially those with low final dFLC of <10mg/L) show LS% improvements; reinforcing the need for deep haematological responses in patients with cardiac AL amyloidosis. An absolute improvement in LS% of -1.5% is associated with improvement in OS and defines a new group with a better prognosis than those with a traditional cardiac response alone following therapy for AL amyloidosis. These data suggest that LS % could be incorporated into both a future staging system for patients with newly diagnosed AL amyloidosis at baseline and into the criteria used to define cardiac response. Changes in LS% hold promise to be useful endpoints for clinical trials of agents that target removal of amyloid deposits.

Acknowledgements

OCC, CJW and ADW conceived the study. AI, SO, BP and BD provided analysis of echocardiograms. OC, RM and DF collected the data. OC, CJW, RHF and ADW wrote the manuscript. All authors provided critical input into the manuscript and approved the final version prior to publication.

Disclosure

We thank Caelum Biosciences for support with data collection costs for this study

The authors have no competing financial interests

Median (range) / n(%)	Cardiac Involvement (n=628)	No Cardiac Involvement (n=287)
Age (years)	68 (32-89)	71 (45-92)
Male	61.1%	46.0%
NYHA class:		
1	99 (15.8)	124 (44.3)
2	333 (53.0)	113 (39.4)
3	98 (15.6)	10 (3.5)
4	3 (0.5)	1 (0.4)
Not recorded	95 (15.1)	39 (13.6)
NT-proBNP (ng/L)	4076 (93-93796)	329 (12-19315)
Mayo Stage:		
I	7 (1.1)	133 (46.3)
II	189 (30.1)	105 (36.6)
IIIa, NT-proBNP ≤8500 ng/L	301 (47.9)	33 (11.5)
IIIb, NT-proBNP >8500 ng/L	117 (18.6)	5 (1.7)
Not recorded	14 (2.2)	11 (3.8)
Median SBP (mmHg)	116 (76-194)	130 (88-190)
Renal involvement	378 (60.2)	245 (85.4)
No. patients on dialysis	24 (3.8)	24 (8.4%)
No. organs involved	2 (1-5)	1 (1-3)
dFLC (mg/L)	236 (2.5-15898)	105 (0-3822)

Table 1: Baseline Characteristics

Abbreviations: NYHA: New York Heart Association class, NT-proBNP: N-terminal probrain natriuretic peptide; SBP: systolic blood pressure; dFLC: difference between free light chains.

Variable	Hazard ratio	95% CI	P value
II	Reference		<0.0001
IIIa	1.76	1.32-2.36	<0.0001
IIIb	2.85	2.05-3.97	<0.0001
LS ≤ -17.0%	Reference		<0.0001
LS -10.3 to - 16.9%	1.71	1.19-2.46	0.004
LS ≥-10.2%	2.73	1.89-3.93	<0.0001

Table 2: Multivariate analysis of Mayo stage criteria incorporating LS%

Abbreviations: CI: confidence interval; LS%: global longitudinal strain percentage.

Parameter: (n=342)	CR (n=82)		P value	VGPR (n=143)		P value	PR (n=71)		P value	NR (n=29)		P Value
	BL	12m		BL	12m		BL	12m		BL	12m	
LV Wall thickness	14.5	14.5	0.90	15.0	15.2	0.07	**15.2**	**15.5**	**0.04**	**14.5**	**15.3**	**0.0003**
LVEF	55.4	55.2	0.82	**56.8**	54.5	**0.0002**	**56.8**	**54.3**	**0.01**	**55.3**	**50.0**	**0.007**
LV LS%	**-13.7**	**-14.6**	**0.04**	-13.9	-13.8	0.74	-13.7	-13.0	0.10	**-14.6**	**-12.6**	**0.006**
Basal Lateral LS %	**-9.1**	**-10.3**	**0.007**	-9.0	-9.3	0.47	-8.8	-8.1	0.17	-9.8	-8.3	0.053
Basal Septal LS %	**-6.6**	**-7.9**	**0.007**	-7.0	-7.2	0.38	-6.5	-6.1	0.34	-7.6	-6.9	0.30
Apex Lateral LS %	-21.3	-20.5	0.28	-20.7	-19.5	0.07	-20.6	-19.4	0.06	**-21.6**	**-18.3**	**0.02**
Apex Septal LS %	-21.7	-22.3	0.33	-22.2	-21.2	0.06	-21.6	-20.7	0.16	**-23.0**	**-20.5**	**0.04**

Table 3: Median echocardiographic parameters in patients with cardiac AL amyloidosis based upon haematological response at 12 months

*17 patients not evaluable for haematological response

Abbreviations: CR: complete response; VGPR: very good partial response; PR: partial response; NR: no response; LV: Left ventricle (LV), LVEF: Left-ventricular ejection fraction, LS %: global longitudinal strain %.

	n	Baseline LS%	12 months LS %	Change in LS%	P value
dFLC<10	132	-13.8	-14.6	-0.8	0.01
dFLC 10-40	193	-13.8	-13.0	+0.8	0.003
CR + dFLC<10	72	-13.8	-14.9	-1.1	0.02
Not-CR + dFLC<10	60	-13.9	-14.3	-0.4	0.32

Table 4: Global longitudinal strain at baseline and 12 months according to 6 month dFLC response

Abbreviations: LS %: Longitudinal strain %; dFLC: difference between involved and uninvolved free light chains; CR: complete response.

n=236*	Cardiac Responders (n=133)				Cardiac Non-responders (n=70)			
	BL	12m	24m	P value	BL	12m	24m	P value
LV wall thickness	15.0	15.1	15.0	0.50	14.8	15.0	15.1	**0.02**
LVEF	55.5	55.5	55.6	0.95	57.7	53.1	53.5	**<0.0001**
LV LS%	-12.8	-13.7	-14.4	**<0.0001**	-14.4	-13.0	-13.4	**0.004**
Baso-lateral LS %	-7.9	-9.5	-10.1	**0.0002**	-9.7	-8.9	-8.9	0.20
Baso-septal LS %	-6.1	-6.8	-7.6	**<0.0001**	-7.2	-6.5	-7.2	0.08
Apex Lateral LS %	-19.4	-19.9	-20.9	**0.04**	-21.2	-19.2	-19.0	**0.01**
Apex Septal LS %	-20.6	-21.1	-22.1	**0.01**	-22.8	-20.8	-21.0	**0.01**

Table 5: Echocardiographic parameters in patients with cardiac involvement at baseline, 12 and 24 months according to cardiac response

*33 patients excluded from analysis due to lack of evaluable cardiac response

Abbreviations: LV: Left ventricle; LVEF: Left-ventricular ejection fraction; LS %: Longitudinal strain %.

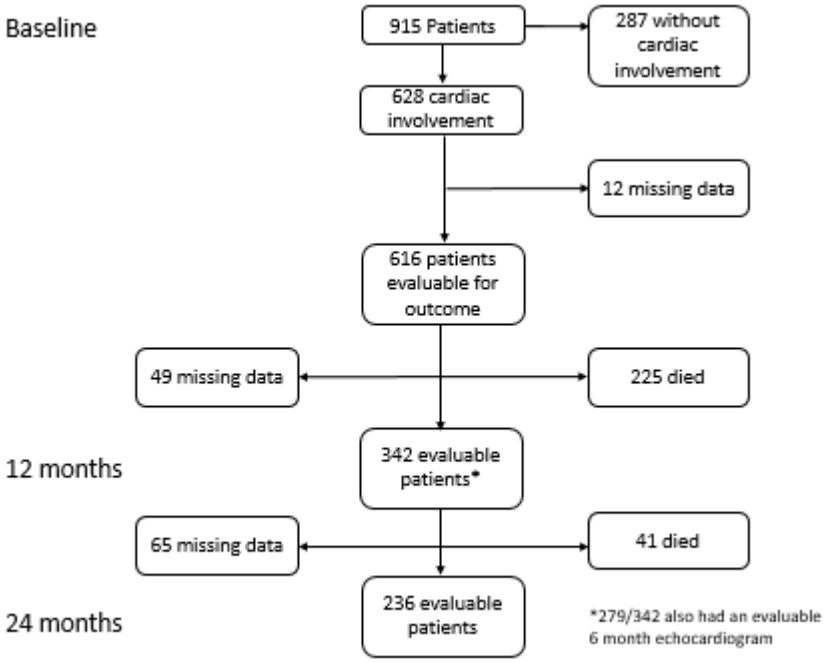


Figure 1: Flow diagram of patient recruitment and inclusion within response assessment based on availability of echocardiographic data

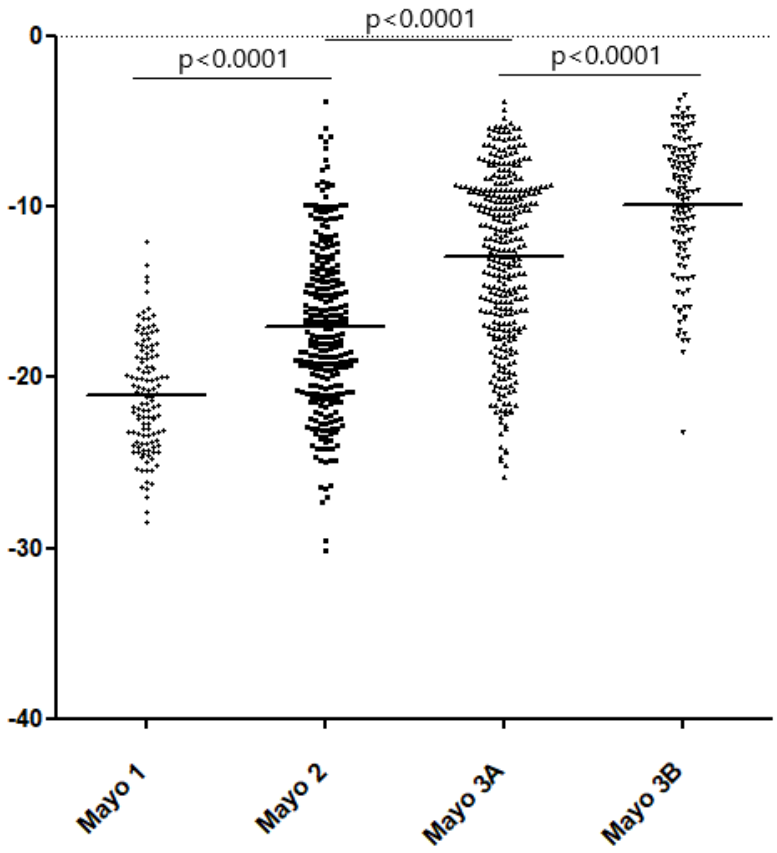


Figure 2: Difference in mean baseline LS% between Mayo stages

A)

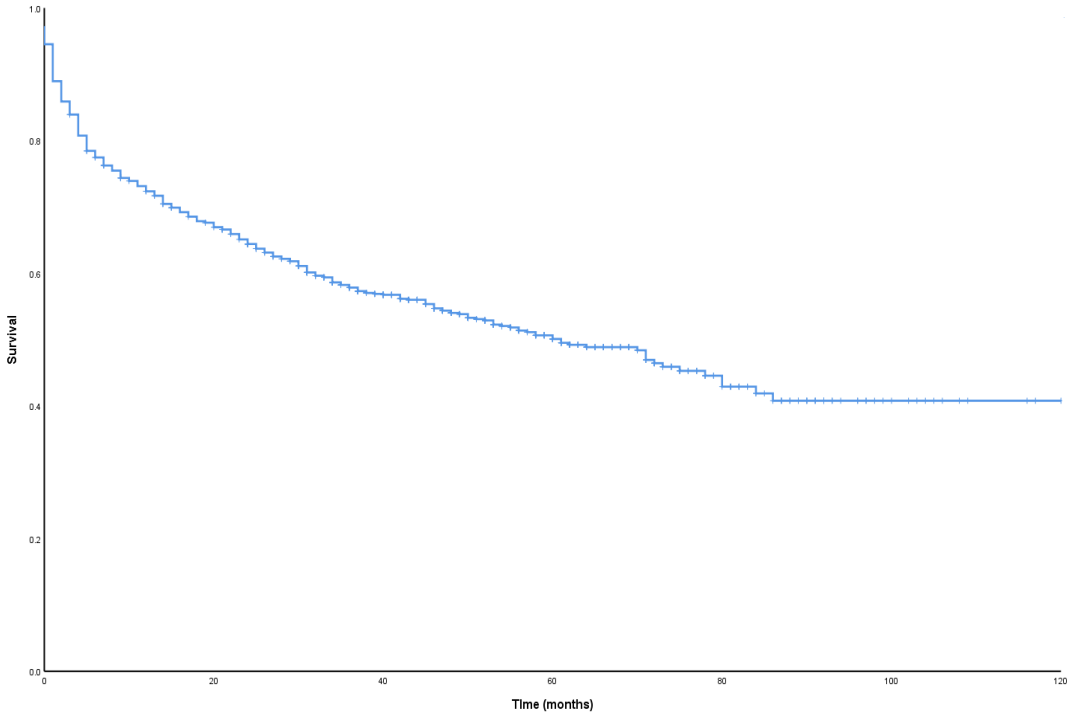
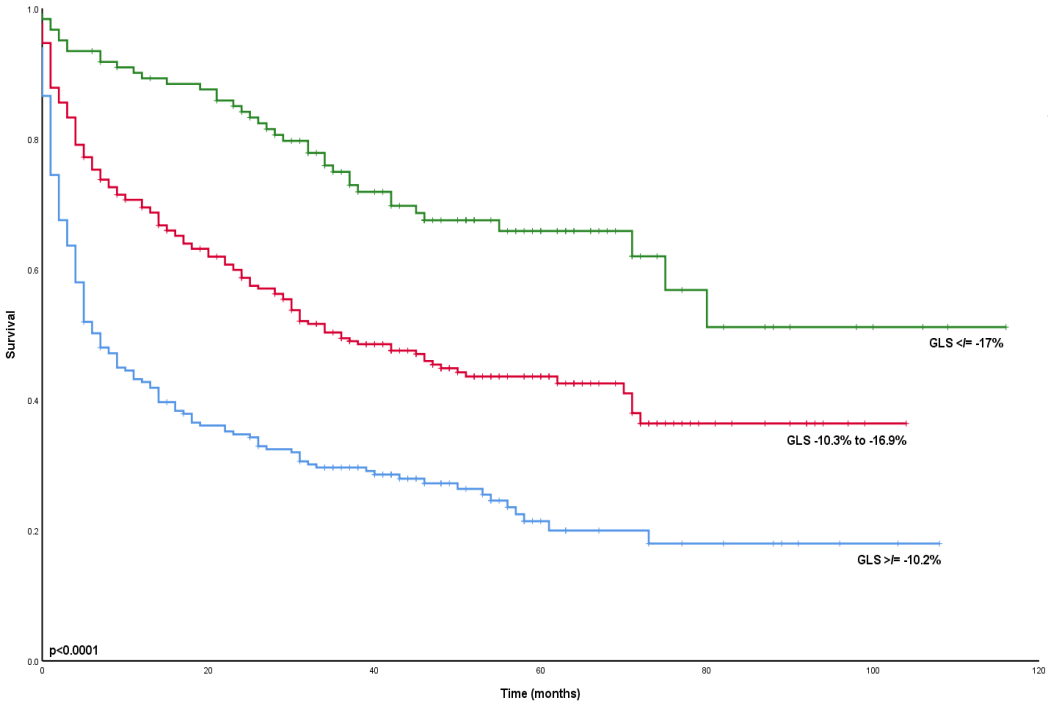
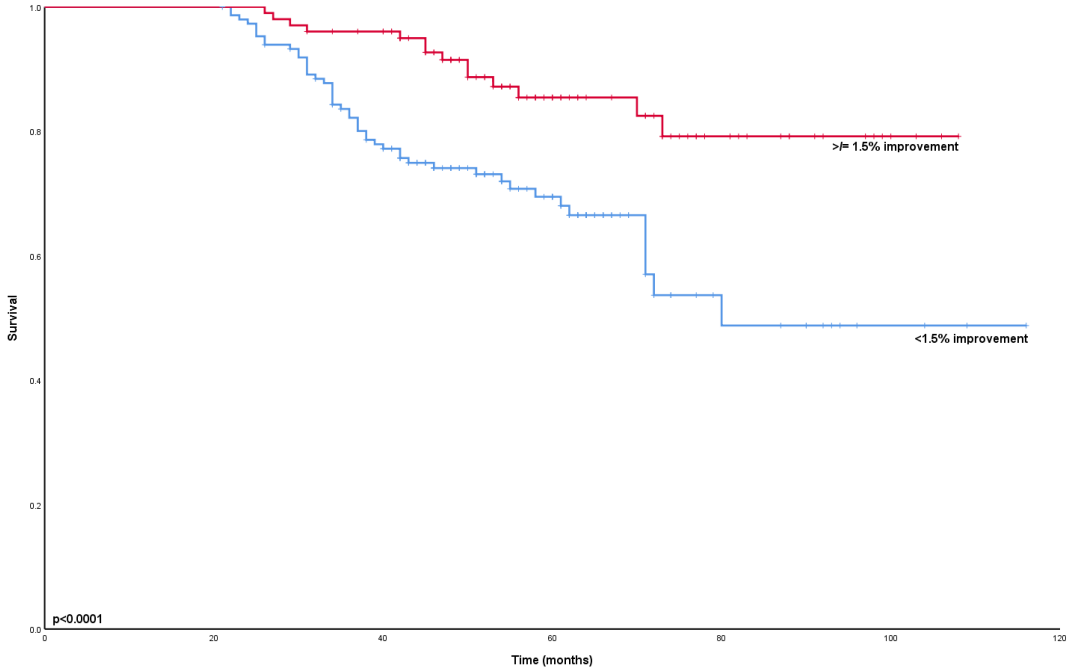


Figure 3: Survival in patients with newly diagnosed systemic AL amyloidosis

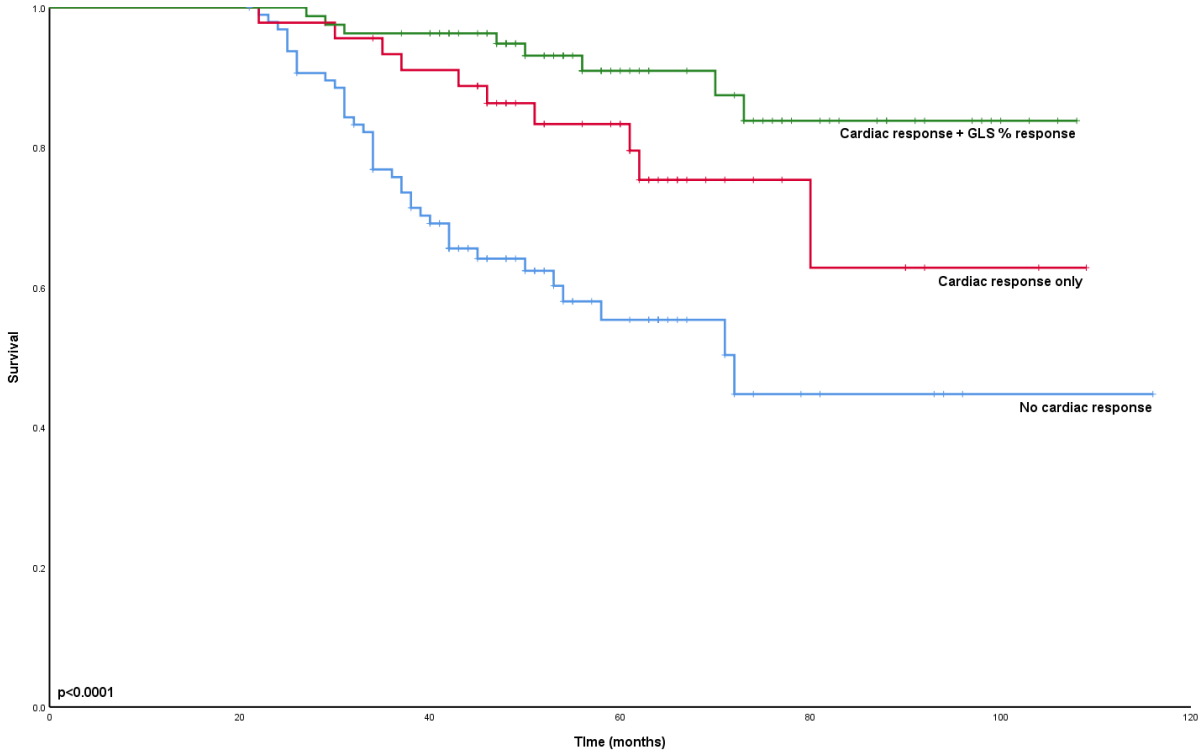
A) Overall survival in the full cohort showing median OS – 61 (95% CI: 49.9-72.1) months.



B) Overall survival by baseline LS% showing patient with presenting LS% ≤-17.0% - OS was not reached, LS% between -16.9 - -10.3% - median OS - 36 (95% CI: 24.9-47.1) months and LS% ≥-10.2% - median OS - 7 (95% CI: 4.5-9.5) months (p<0.0001)



C) Overall survival by 1.5% improvement in LS% at 24 months showing patients with a 1.5% LS% improvement – OS was not reached whilst LS% improvement <1.5% - OS was 80 (95% CI: 74.6-90.5) months (p<0.0001)



D) Overall survival by both 1.5% LS% response and traditional cardiac response at 24 months showing patients with both a 1.5% LS% improvement and cardiac response – OS not reached – lives longer than those achieving a cardiac response alone – OS also not reached ($p<0.0001$). Patients not achieving a cardiac response had an OS 72 (95% CI: 50.5-93.5) months. ($p<0.0001$)

Supplementary Table 1: Median baseline echocardiographic parameters by cardiac involvement

	All (n=915)	Cardiac Involvement* (n=628)	No Cardiac Involvement* (n=287)	P value
LV wall thickness (cm)	14 (7 – 25)	15 (9-25)	11 (7-15)	**<0.0001**
LV EF (%)	60 (16 – 75)	56 (16-75)	60 (16-73)	**<0.0001**
LV LS (%)	-15.2 (-30.1 - -3.4)	-12.2 (-27.0 - -3.4)	-18.4 (-30.1 - -7.8)	**<0.0001**
Basal Lateral LS (%)	-10 (-31 - +9)	-8.0 (-31 - -1)	-14.0 (-30 - -1)	**<0.0001**
Basal Septal LS (%)	-7 (-26 - +7)	-5.0 (-21 - -1)	-11.0 (-26 - -3)	**<0.0001**
Apical Lateral LS (%)	-22 (-43 - +18)	-19.0 (-42 - -3)	-23.0 (-43 - -9)	**<0.0001**
Apical septal LS (%)	-23 (-45 - +31)	-20.0 (-43 - -4)	-24.8 (-45 - -9)	**<0.0001**

*Patients with LV wall thickness <12mm who had a cardiac magnetic resonance imaging (CMR) scan performed, which demonstrated characteristic appearances of cardiac amyloidosis were included.

Abbreviations: LV: Left ventricle; LVEF: Left-ventricular ejection fraction; LS %: global longitudinal strain %.

Supplementary Table 2: B: Median baseline echocardiographic parameters by Mayo staging

	Mayo 2	Mayo 3A	Mayo 3B	P value
LV wall thickness	13.1	14.8	15.6	**<0.0001**
LVEF	60.2	55.9	51.9	**<0.0001**
LV LS%	-17.4	-13.7	-11.5	**<0.0001**
Basal Lat LS %	-13.6	-8.6	-6.9	**<0.0001**
Basal Sep LS %	-10.1	-6.7	-5.3	**<0.0001**
Apex Lat LS %	-24.2	-21.4	-17.5	**<0.0001**
Apex Sep LS %	-25.3	-21.5	-19.5	**<0.0001**

Abbreviations: LV: Left ventricle; LVEF: Left-ventricular ejection fraction; LS %: global longitudinal strain %.

Supplementary Table 3: Univariate analysis of the impact of echocardiographic parameters on survival in patients with cardiac involvement

Variable	HR	95% CI	P value
LV Wall thickness	1.11	1.06-1.15	**<0.0001**
LVEF	0.96	0.95-0.97	**<0.0001**
LS%	1.12	1.09-1.14	**<0.0001**
Bas Lateral LS%	1.08	1.05-1.10	**<0.0001**
Bas Septal LS%	1.11	1.08-1.14	**<0.0001**
Apex Lateral LS%	1.06	1.05-1.08	**<0.0001**
Apex Septal LS%	1.06	1.05-1.08	**<0.0001**

Abbreviations: LV: Left ventricle; LVEF: Left-ventricular ejection fraction; LS %: global longitudinal strain %.

Supplementary Table 4: Changes in echocardiographic parameters in patients with cardiac AL amyloidosis based upon haematological response at 6 months

Parameter: (n=279*)	CR (n=67)		P value	VGPR (n=123)		P value	PR (n=57)		P value	NR (n=21)		P Value
	BL	6m		BL	6m		BL	6m		BL	6m	
LV Wall thickness	14.9	14.9	0.76	15.1	15.1	0.75	**15.1**	**15.4**	**0.04**	**14.5**	**15.1**	**0.04**
LVEF	55.3	54.2	0.20	**56.4**	**54.6**	**0.004**	**56.3**	**54.1**	**0.01**	55.3	52.1	0.10
LV LS%	-13.5	-13.4	0.82	-13.4	-13.5	0.74	-13.8	-13.1	0.15	**-14.6**	**-12.4**	**0.0005**
Basal Lateral LS %	-8.8	-8.6	0.68	-8.6	-8.9	0.38	**-8.7**	**-7.6**	**0.01**	-9.3	-8.4	0.15
Basal Septal LS %	-6.3	-6.4	0.77	-6.7	-6.7	0.93	-6.6	-6.2	0.24	-8.0	-6.7	0.10
Apex Lateral LS %	**-21.3**	**-19.8**	**0.03**	-20.1	-20.1	0.93	-20.8	-20.3	0.57	**-21.8**	**-18.2**	**0.02**
Apex Septal LS %	-21.6	-20.6	0.15	-21.6	-20.9	0.37	-21.7	-21.1	0.54	**-23.1**	**-19.0**	**0.006**

*11 patients not evaluable for haematological response

Abbreviations: CR: complete response; VGPR: very good partial response; PR: partial response; NR: no response; LV: Left ventricle (LV), LVEF: Left-ventricular ejection fraction, LS %: global longitudinal strain %.

References

1. Wechalekar AD, Gillmore JD, Hawkins PN. Systemic amyloidosis. Lancet. 2016;387(10038):2641-54.
2. Muchtar E, Gertz MA, Kumar SK, Lacy MQ, Dingli D, Buadi FK, et al. Improved outcomes for newly diagnosed AL amyloidosis between 2000 and 2014: cracking the glass ceiling of early death. Blood. 2017;129(15):2111-9.
3. Chacko L, Martone R, Cappelli F, Fontana M. Cardiac Amyloidosis: Updates in Imaging. Curr Cardiol Rep. 2019;21(9):108.
4. Dispenzieri A, Gertz MA, Kyle RA, Lacy MQ, Burritt MF, Therneau TM, et al. Serum cardiac troponins and N-terminal pro-brain natriuretic peptide: a staging system for primary systemic amyloidosis. J Clin Oncol. 2004;22(18):3751-7.
5. Wechalekar AD, Schonland SO, Kastritis E, Gillmore JD, Dimopoulos MA, Lane T, et al. A European collaborative study of treatment outcomes in 346 patients with cardiac stage III AL amyloidosis. Blood. 2013;121(17):3420-7.
6. Gertz MA, Comenzo R, Falk RH, Fermand JP, Hazenberg BP, Hawkins PN, et al. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. American journal of hematology. 2005;79(4):319-28.
7. Rapezzi C, Fontana M. Relative Left Ventricular Apical Sparing of Longitudinal Strain in Cardiac Amyloidosis: Is it Just Amyloid Infiltration? JACC Cardiovasc Imaging. 2019;12(7 Pt 1):1174-6.
8. Buss SJ, Emami M, Mereles D, Korosoglou G, Kristen AV, Voss A, et al. Longitudinal left ventricular function for prediction of survival in systemic light-chain amyloidosis: incremental value compared with clinical and biochemical markers. J Am Coll Cardiol. 2012;60(12):1067-76.
9. Salinaro F, Meier-Ewert HK, Miller EJ, Pandey S, Sanchorawala V, Berk JL, et al. Longitudinal systolic strain, cardiac function improvement, and survival following treatment of light-chain (AL) cardiac amyloidosis. Eur Heart J Cardiovasc Imaging. 2017;18(9):1057-64.
10. Barros-Gomes S, Williams B, Nhola LF, Grogan M, Maalouf JF, Dispenzieri A, et al. Prognosis of Light Chain Amyloidosis With Preserved LVEF: Added Value of 2D Speckle-Tracking Echocardiography to the Current Prognostic Staging System. JACC Cardiovasc Imaging. 2017;10(4):398-407.
11. Pun SC, Landau HJ, Riedel ER, Jordan J, Yu AF, Hassoun H, et al. Prognostic and Added Value of Two-Dimensional Global Longitudinal Strain for Prediction of Survival in Patients with Light Chain Amyloidosis Undergoing Autologous Hematopoietic Cell Transplantation. J Am Soc Echocardiogr. 2018;31(1):64-70.
12. Milani P, Dispenzieri A, Scott CG, Gertz MA, Perlini S, Mussinelli R, et al. Independent Prognostic Value of Stroke Volume Index in Patients With Immunoglobulin Light Chain Amyloidosis. Circ Cardiovasc Imaging. 2018;11(5):e006588.
13. Liu D, Hu K, Herrmann S, Cikes M, Ertl G, Weidemann F, et al. Value of tissue Doppler-derived Tei index and two-dimensional speckle tracking imaging derived longitudinal strain on predicting outcome of patients with light-chain cardiac amyloidosis. Int J Cardiovasc Imaging. 2017;33(6):837-45.
14. Geenty P SS, Deshmukh T, Brown P, Boyd A, Kwok F, Richards D, Altman M, Stewart G, Thomas L. The use of echocardiographic parameters to predict clinical outcomes in al-amyloidosis cardiomyopathy. European Heart Journal - Cardiovascular Imaging 2020;21.
15. Liu D, Hu K, Stork S, Herrmann S, Kramer B, Cikes M, et al. Predictive value of assessing diastolic strain rate on survival in cardiac amyloidosis patients with preserved ejection fraction. PLoS One. 2014;9(12):e115910.
16. Takemura G, Takatsu Y, Doyama K, Itoh H, Saito Y, Koshiji M, et al. Expression of atrial and brain natriuretic peptides and their genes in hearts of patients with cardiac amyloidosis. J Am Coll Cardiol. 1998;31(4):754-65.

17. Bhutani D LS, Eisenberger A, Maurer MS, Shames S, Goldsmith J, Lentzsch S. . Improvement in Global Longitudinal Strain (GLS) Correlates with NT-Probnp Response in Patients with Cardiac Amyloidosis Treated on a Phase 1b Study of Anti-Amyloid Mab Cael-101 American Society of Haematology; San Diego, USA2018.
18. Comenzo RL, Reece D, Palladini G, Seldin D, Sanchorawala V, Landau H, et al. Consensus guidelines for the conduct and reporting of clinical trials in systemic light-chain amyloidosis. Leukemia. 2012;26(11):2317-25.
19. Palladini G, Dispenzieri A, Gertz MA, Kumar S, Wechalekar A, Hawkins PN, et al. New criteria for response to treatment in immunoglobulin light chain amyloidosis based on free light chain measurement and cardiac biomarkers: impact on survival outcomes. J Clin Oncol. 2012;30(36):4541-9.
20. Manwani R, Cohen O, Sharpley F, Mahmood S, Sachchithanantham S, Foard D, et al. A prospective observational study of 915 patients with systemic AL amyloidosis treated with upfront bortezomib. Blood. 2019.
21. Chacko L, Martone R, Bandera F, Lane T, Martinez-Naharro A, Boldrini M, et al. Echocardiographic phenotype and prognosis in transthyretin cardiac amyloidosis. Eur Heart J. 2020.
22. Liu D, Hu K, Niemann M, Herrmann S, Cikes M, Stork S, et al. Effect of combined systolic and diastolic functional parameter assessment for differentiation of cardiac amyloidosis from other causes of concentric left ventricular hypertrophy. Circulation Cardiovascular imaging. 2013;6(6):1066-72.
23. Phelan D, Collier P, Thavendiranathan P, Popovic ZB, Hanna M, Plana JC, et al. Relative apical sparing of longitudinal strain using two-dimensional speckle-tracking echocardiography is both sensitive and specific for the diagnosis of cardiac amyloidosis. Heart. 2012;98(19):1442-8.
24. Kotecha T, Martinez-Naharro A, Treibel TA, Francis R, Nordin S, Abdel-Gadir A, et al. Myocardial Edema and Prognosis in Amyloidosis. J Am Coll Cardiol. 2018;71(25):2919-31.
25. Wechalekar AD, Gillmore JD, Bird J, Cavenagh J, Hawkins S, Kazmi M, et al. Guidelines on the management of AL amyloidosis. Br J Haematol. 2015;168(2):186-206.
26. Gertz MA. Immunoglobulin light chain amyloidosis diagnosis and treatment algorithm 2018. Blood Cancer J. 2018;8(5):44.
27. Vo T NT, Chen A, French J, Otton J, Mussap C, Richards D, Dimitri H, Thomas L. Left ventricular global longitudinal strain recovery predicts scar size reduction and systolic remodelling post ST-elevation myocardial infarction European Heart Journal 2019;40.
28. Manwani R, Cohen O, Sharpley F, Mahmood S, Sachchithanantham S, Foard D, et al. A prospective observational study of 915 patients with systemic AL amyloidosis treated with upfront bortezomib. Blood. 2019;134(25):2271-80.