# Cardiorenal AL amyloidosis: risk stratification and outcomes based upon cardiac and renal biomarkers

**Running Title: Cardiorenal syndrome in AL amyloidosis**

Tamer Rezk Bsc[1,2], Helen J Lachmann MD[1], Marianna Fontana PhD[1], Ana Martinez Naharro MD[1], Sajitha Sachchithanantham MD[1], Shameem Mahmood MD[1], Aviva Petrie MSc [3], Carol J Whelan MD[1], Jennifer H Pinney MD[1], Darren Foard[1], Thirusha Lane PhD[1], Taryn Youngstein Bsc[1], Ashutosh D Wechalekar MD[1], Philip N Hawkins PhD[1], Julian D Gillmore PhD[1]

[1]National Amyloidosis Centre, [2]UCL Centre for Nephrology, Division of Medicine, University College London & [3]UCL Eastman Dental Institute

Correspondence: Professor JD Gillmore, National Amyloidosis Centre, Division of Medicine, University College London, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK

Tel: +44 (0)20 7433 2726     E-mail: j.gillmore@ucl.ac.uk

Fax: +44 (0)20 74332844

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## Summary

Systemic AL amyloidosis is a cause of type 5 cardiorenal syndrome. Response to treatment is currently reported according to organ-specific amyloidosis consensus criteria (ACC) which are not validated in cardiorenal AL amyloidosis. Of 1000 patients prospectively enrolled into the UK ALchemy study, 318 (32%) had combined cardiac and renal amyloidotic organ dysfunction at diagnosis among whom 199 (63%) died; median survival by Kaplan Meier analysis was 18.5 months. Fifty (16%) patients required renal replacement therapy (RRT). At diagnosis, independent predictors of both death and dialysis were N-terminal pro-b-type natriuretic peptide (NT-proBNP) >8500ng/L (HR 3.30, p<0.001; HR 3.00, p<0.001), and estimated glomerular filtration rate (eGFR) <30ml/min/1.73 m$^2$ (HR 1.89, p=0.011; HR 6.37, p<0.001). At 6 months, an increase in NT-proBNP of >30% and a reduction in eGFR of $\geq$ 25% were independent predictors respectively of death (HR 2.17, p=0.009) and dialysis (HR 3.07, p=0.002). At 12 months, an increase in NT-proBNP of >30% was highly predictive of death (HR 3.67, p<0.001) and dialysis (HR 2.85, p=0.010), whereas ACC renal response was predictive of neither. Cardiorenal AL amyloidosis is associated with high early mortality. Outcomes are dictated by NT-proBNP and eGFR at diagnosis rather than proteinuria, and thereafter predominantly by changes in NT-proBNP concentration.

## Key Words:

Cardiology, Cardiorenal, Amyloidosis, NT-proBNP, dialysis

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## Introduction

The amyloidoses are disorders of protein folding, in which a variety of proteins misfold and aggregate into fibrils that accumulate in tissues and disrupt organ function.(Palladini, *et al* 2003) Immunoglobulin light chain (AL) amyloidosis is caused by deposition of fibrils derived from monoclonal immunoglobulin light chains and is the most common and serious type of systemic amyloidosis.(Pepys 2006) Renal and cardiac involvement respectively are present in approximately 70% and 50% of patients with systemic AL amyloidosis at diagnosis. Renal involvement manifests with nephrotic syndrome and progressive renal impairment,(Palladini, *et al* 2012) and cardiac involvement with congestive cardiac failure. Progression to end-stage renal disease (ESRD) is one of the main determinants of morbidity in AL amyloidosis, whilst presence and severity of cardiac amyloidosis is the main determinant of mortality.(Kyle, *et al* 1986, Wechalekar, *et al* 2013)

Cardiorenal syndrome is increasingly being recognised as a distinct clinical entity. Recent classifications have separated cardiorenal syndrome into 5 types; acute heart failure leading to acute kidney injury (Type 1), chronic abnormalities in cardiac function leading to progressive chronic kidney disease (CKD) (Type 2), acute deterioration in renal function leading to acute cardiac dysfunction (Type 3), chronic kidney disease contributing to progressive cardiac impairment (Type 4) and systemic diseases affecting both the heart and kidneys in both the acute and chronic setting (Type 5). Systemic AL amyloidosis is one of the main causes of type 5 cardiorenal syndrome.(Ronco, *et al* 2008, Soni, *et al* 2012)

The main established determinant of outcome in systemic AL amyloidosis is the depth of serum free light chain response to chemotherapy.(Mahmood, *et al* 2014, Palladini, *et al* 2012) Patients who achieve either a very good partial response (VGPR) or a complete response (CR) of the underlying hematologic condition have the highest chance of subsequent

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improvement in amyloidotic organ dysfunction, termed an 'organ' response.(Palladini, *et al* 2012, Palladini, *et al* 2014, Pinney, *et al* 2011)  In 2005, amyloidosis consensus criteria (ACC) were established to define individual organ involvement in systemic AL amyloidosis as well as individual organ responses.

In 2014, renal staging and 'early' renal response criteria (at 6 months) using eGFR and proteinuria to predict renal survival were developed for renal AL amyloidosis.  Despite concurrent cardiac amyloidosis among 70% of the cohort that were employed to establish these criteria, alternative biomarkers such as N-terminal pro-b-type natriuretic peptide (NT-proBNP) were not included in the analysis.(Palladini, *et al* 2014)  Whilst early renal response was highly predictive of renal survival, it was not predictive of overall survival; furthermore, a composite endpoint including first of either death or dialysis was not explored. The current ACC response criteria have not therefore been specifically validated in patients with cardiorenal syndrome due to AL amyloidosis and have not been examined with respect to the key outcome measures in this population of death, dialysis or the composite endpoint of either death or dialysis.

We report renal and patient outcomes among 318 patients with systemic AL amyloidosis and cardiorenal syndrome who were prospectively enrolled into the UK AL Amyloidosis Chemotherapy (ALchemy) study between 2009 and 2016, in relation to their baseline clinical variables and stratify them at different timepoints according to their amyloidotic organ function.

## Patients and Methods

### Patients

At the time of censor, 1000 patients with newly diagnosed AL amyloidosis had been enrolled into the prospective ALchemy study at the UK National Amyloidosis Centre (NAC). Cardiorenal syndrome was defined by the combination of more than 0.5g/24 hr of non-Bence Jones proteinuria and presence of cardiac involvement by echocardiography according to amyloidosis consensus criteria.(Gertz, *et al* 2005) In rare cases in which echocardiography was suggestive but not conclusive with respect to presence of cardiac amyloidosis, it was supplemented by assessment of late gadolinium enhancement images on CMR, as previously defined.(Fontana, *et al* 2015, Maceira, *et al* 2005) All analyses presented in this manuscript were performed in the cohort of patients with systemic AL amyloidosis who satisfied the above criteria for cardiorenal syndrome.

All patients underwent protocolized assessments at the NAC at baseline, 3, 6 and 12 months and subsequently at 6 monthly intervals, each comprising a complete clinical evaluation including volume status evaluation and medication review, as well as serum and urine biochemistry including assessment of renal and liver function, NT-proBNP, echocardiography, NYHA functional class, 6 minute walk test (6MWT), SAP scintigraphy,(Hawkins, *et al* 1990) and assessment of hematologic disease by serum free light chain (FLC) assay along with serum and urine immunofixation electrophoresis.

All patients were managed in accordance with the Declaration of Helsinki and provided written informed consent for study entry (REC reference 09/H0715/58) and publication of their data.

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## Assessment of Hematologic response

Details and doses of chemotherapy regimens were collected. All patients had serial FLC concentration prospectively monitored on blood samples obtained monthly during periods of chemotherapy treatment, and 1-3 monthly (depending on depth and stability of response) thereafter. Healthy polyclonal serum FLC concentration increases progressively through advancing stages of chronic kidney disease (CKD)(Hutchison, *et al* 2008) which hinders the monitoring of monoclonal light chain disorders. In this study, the value of the FLC monoclonal component was estimated by subtracting the concentration of the uninvolved light chain from that of the amyloidogenic light chain to obtain the FLC difference (dFLC), a strategy previously validated in multiple myeloma and AL amyloidosis.(Dispenzieri, *et al* 2008, Pinney, *et al* 2011)

The FLC response to chemotherapy was determined according to previously validated ACC.(Comenzo, *et al* 2012b) Since ACC define "evaluable" patients as those with a pre-treatment (baseline) dFLC of >50 mg/l, thus excluding 27/318 (9%) patients in this cohort, the percentage of the baseline dFLC that remained at the time of analysis (percentage method), also validated in AL amyloidosis,(Pinney, *et al* 2011, Rezk, *et al* 2017) was additionally used to determine response to chemotherapy. A very good partial response (VGPR) was defined according to the ACC as an absolute dFLC of <40mg/L and by the percentage method as a ≥90% reduction of pre-treatment dFLC remaining after chemotherapy, as previously described.(Pinney, *et al* 2011, Rezk, *et al* 2017)

## Assessment of Organ Response

Organ responses were defined according to ACC;(Comenzo, *et al* 2012a) cardiac progression as an increase in NT-proBNP of >30% and >300 ng/L and cardiac response as a reduction of NT-proBNP of >30% (and >300 ng/L decrease in patients with baseline NT-proBNP

≥ 650 ng/L).  Renal progression was defined as a ≥ 25% reduction in eGFR and or a ≥ 50% increase in proteinuria and renal response was defined as a ≥ 50% reduction in proteinuria without a ≥ 25% reduction in eGFR.  Additionally, 'early' renal progression was defined at six months from baseline as a ≥ 25% reduction in eGFR and 'early' renal response was defined at six months from baseline as a ≥ 30% reduction in proteinuria without a ≥ 25% reduction in eGFR, according to previously published amyloidosis consensus criteria.(Palladini, *et al* 2014)

**Patient and Renal Survival**

Overall survival (OS) was defined as the time from baseline diagnostic evaluation at the NAC to patient death and was evaluated in all 318 patients.  Renal survival was defined as the time from baseline diagnostic evaluation at the NAC to requirement for renal replacement therapy (RRT).  For the analyses of renal survival, patients who were already established on RRT (n=7) at the time of their baseline evaluation were excluded, and those who died without requiring RRT were censored at the time of death.  For analyses of time to the composite endpoint of death or dialysis, patients who were on RRT at baseline were excluded and an event was recorded as the first of either death or dialysis.  Patient follow up was censored on 1st September 2016.

**Statistical Analysis**

Median patient survival, renal survival, and survival to the composite endpoint of 'dialysis or death', were determined by Kaplan-Meier (KM) survival analysis using GraphPad Prism v5.03 software.  All analyses examining clonal or organ response at set time points were performed as landmark analyses. Univariable Cox proportional hazards regression analysis was used to investigate the factors associated with the endpoints of death, dialysis, or the composite

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endpoint of 'dialysis or death' at baseline and at relevant time points for each of the three endpoints.Ordinal variables were tested for trend using the Peto-Peto-Prentice modification. Multivariable Cox regression analysis was subsequently used to investigate factors independently associated with each of the three endpoints at the relevant time points. Stata software (StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC) was used for regression analyses. A significance level of 0.05 was used for all hypothesis tests.

## Results

### Baseline Characteristics and Patient Survival

Type 5 cardiorenal syndrome, defined by the combination of renal amyloidosis (more than 0.5g/24 hr of non-Bence Jones proteinuria by ACC organ involvement criteria) and cardiac amyloidosis (>12 mm IVSd thickness on echocardiography without an alternative cause), was present in 318 patients at the time of diagnosis. Histological proof of amyloid was established in all patients including renal biopsy evidence of AL amyloid deposits in 150/318 (47%) cases and endomyocardial biopsy evidence of AL amyloid deposits in a further 6 patients; no patient in the whole cohort of 318 patients had either a cardiac or renal biopsy which failed to show amyloid. Among 162/318 cases who did not have endomyocardial or renal biopsy 'proof' of AL amyloid deposits, all had clear evidence of cardiac and renal amyloidosis on the basis of existing ACC accompanied by AL amyloid deposits in a biopsy specimen from an alternative site. In 310/318 patients, cardiac amyloidosis was definitively diagnosed by echocardiography (although frequently supplemented by characteristic CMR findings) but in the remaining 8 cases in which echocardiography was not definitively diagnostic of cardiac amyloidosis, its presence was confirmed by the finding of characteristic late gadolinium enhancement by CMR.

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Of the 168 patients who did not undergo a renal biopsy, 80/168 (47%) had nephrotic range proteinuria (≥ 3g/24hr).

Baseline demographics and clinical characteristics of the whole cohort of 318 patients with cardiorenal syndrome are listed in Table 1. Median age at diagnosis was 66 years with M:F ratio of 1:1. Median eGFR was 55 ml/min/1.73 m$^2$ (range 10-100) with a median 24 hour urinary protein leak of 4.6 grams (range 0.5-26.8) and median serum albumin of 30 g/L (14-49). Mayo disease Stage was 1, 2 and 3 in 4 (1%), 92 (29%) and 222 (70%) of patients respectively. Median NT-proBNP was 5568 ng/L (range 145-70,295) with 66 (21%) patients having a concentration of >8500 ng/L (defined by amyloidosis staging criteria as Stage 3b disease).(Wechalekar, *et al* 2013) Median follow up by reverse Kaplan-Meier (KM) analysis for the whole cohort was 47.5 months (range 0.1-83.5). A total of 199/318 (63%) patients died with a median overall survival of only 18.5 months by KM analysis. Among the 199 patients who died, median time to death was 5.0 months (range 0.1-54.8); 114/199 (57%) patients died within 6 months and 146/199 (73%) within 12 months of baseline.

Fifty (16%) patients in the whole cohort required RRT during follow up (of whom 7 were already dialysis dependent at baseline). Median time to commencement of dialysis among those 50 patients was 6.6 months (range 0-57.8). Twenty-four patients commenced dialysis within 6 months and 31 within 12 months of baseline. Median overall survival from diagnosis in the cohort of 50 patients who required RRT by KM analysis was 37.4 months, and median survival from commencement of RRT in those 50 patients was 23.0 months.

**Chemotherapy**

Chemotherapy was planned in all 318 patients but was not delivered in 23 (7%) cases, all of whom died prior to starting treatment (median time to death in these 23 patients was 26 days).

Among 295 patients who did receive chemotherapy, 156 (53%) received a bortezomib-based regimen first line, 104 (35%) received a thalidomide-based regimen first line and 35 (12%) received a first line regimen containing neither bortezomib nor thalidomide. Only one patient received upfront high dose melphalan conditioning with autologous stem cell transplantation (ASCT). Median (range) number of chemotherapy cycles administered first line was 4 (1-9).

**Disease-related Predictors of Outcome at Baseline**

Univariable analysis of baseline variables associated with death, dialysis, and the composite endpoint of death or dialysis are listed in Table 2a. Baseline variables that were significantly associated with death were NT-proBNP with a cut of 8500ng/L, (p<0.001), Mayo Stage in three categories (trend test p<0.001), eGFR in six categories (trend test p=0.005), supine systolic blood pressure with a cut of 100mmHg (p<0.001), 6 minute walk test (6MWT) distance with a cut of 300m (p<0.001), NYHA class in four categories (trend test p<0.001) and ECOG performance status in four categories (trend test p<0.001). Baseline variables that were significantly associated with dialysis were NT-proBNP with a cut of 8500ng/L (p<0.001), Mayo Stage in three categories (trend test p<0.001) and eGFR in six CKD categories (trend test p<0.001). Whilst proteinuria, in three categories, demonstrated a significant trend (p=0.036), degree of proteinuria alone was not significantly associated with risk of dialysis, although 'Renal Stage' (eGFR <50ml/min/1.73m$^2$ and/or >5g/24hr of proteinuria)(Palladini, *et al* 2014) was (Stage 3 vs Stage 1; HR 9.40 [3.11-28.37], p<0.001) (Table 2a).

Multivariable analyses with baseline eGFR, NT-proBNP and supine systolic BP as categorical variables revealed NT-proBNP >8500ng/L to be a significant predictor of death (HR 3.01 [2.22-4.10], p<0.001), dialysis (HR 1.98 [1.01-3.88], p=0.045) and the composite endpoint (HR 2.91 [2.16-3.91], p<0.001). Supine systolic BP <100mmHg was an independent

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predictor of death (HR 1.70 [1.23-2.35], p=0.001) and the composite endpoint (HR 1.45 [1.06-1.99], p=0.021), whereas eGFR $<30$ml/min/1.73 m$^2$ was an independent predictor of dialysis (HR 4.86 [2.55-9.25], p$<$0.001) and the composite endpoint (HR 1.73 [1.26-2.39], p=0.001) (Table 2b).

**Outcome in Relation to Hematologic Responses at 3, 6 and 12 months from Baseline**

Univariable analyses of outcome in relation to hematologic response to chemotherapy using both the ACC dFLC method (dFLC $<40$mg/L) and 'percentage' dFLC method are listed in Table 2c. As expected, depth of clonal response was a highly significant predictor of all three outcome measures. There was a highly significant trend across the three categories of percentage dFLC response for death and the composite endpoint at 3, 6 and 12 months (p$\leq$0.003). Whilst there was no evidence of a trend across the three categories of percentage dFLC response at 3 (p=0.088) and 6 (p=0.166) months for the outcome of dialysis; however, achieving a dFLC of $\geq$ 90% at 3, 6 and 12 months was a highly significant (protective) predictor of death (HR 0.15 [0.06-0.33]), dialysis (HR 0.20 [0.05-0.78]) and the composite endpoint (HR 0.31, [0.11-0.84]), compared to $<50$% clonal response.

**Outcomes in Relation to Change in Organ Function at 6 months from Baseline**

In light of the 2014 'early' amyloidosis renal response criteria,(Palladini, *et al* 2014) we assessed renal response at 6 months from baseline and its influence on death, dialysis and the composite endpoint. Univariable analyses revealed that early renal progression (reduction in eGFR of $\geq$ 25%) was predictive of death (HR 1.80 [1.07-3.05], p=0.028), dialysis (HR 3.45 [1.44-8.23], p=0.005) and the composite endpoint of death and dialysis (HR 1.96 [1.16-3.32], p=0.012). Interestingly however, 'early' renal response ($\geq$ 30% reduction in proteinuria

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without a ≥ 25% reduction in eGFR) was not significantly associated with death (HR 0.76 [0.41-1.42], p=0.389), dialysis (HR 0.69 [0.25-1.93], p=0.490) or the composite endpoint (HR 0.80 [0.49-1.47], p=0.478).

In view of the univariable findings, multivariable analyses were performed with increase in NT-proBNP >30%, reduction in eGFR of ≥ 25%, and dFLC response of ≥90% at 6 months from baseline (Table 2b), as categorical variables. Increase in NT-proBNP >30% was predictive of death (HR 2.17 [1.22-3.88], p=0.009) and the composite endpoint (HR 1.70 [1.03-2.81], p=0.037) but not dialysis. Reduction in eGFR of ≥ 25% was highly predictive of dialysis (HR 3.017 [1.51-6.26], p=0.002) and the composite endpoint (HR 1.95 [1.20-3.18], p=0.007) but not death, and dFLC response of ≥90% was (protectively) predictive of death (HR 0.42 [0.24-0.73], p=0.002 and the composite endpoint (HR 0.42 [0.26-0.69], p=0.001) but not dialysis. Landmark analyses were performed to compare overall survival (Figure 1A) among patients who had a >30% increase in NT-proBNP with those who did not have a 30% increase in NT-proBNP at 6 months, and renal survival (Figure 1B) among patients who had a ≥ 25% reduction in eGFR at 6 months with those who did not have a ≥ 25% reduction in eGFR.

**Outcomes in Relation to Change in Organ Function at 12 months from Baseline**

Univariable analyses using the ACC for renal organ response at 12 months were performed. Renal progression (≥ 25% reduction in eGFR and/or a ≥ 50% increase in proteinuria) was not significantly associated with death (HR 1.63 [0.86-3.08], p=0.127) or the composite endpoint (HR 1.70 [0.91-3.20], p=0.096) but was significantly associated with dialysis (HR 3.29 [1.17-9.24], p=0.024). Renal response (≥ 50% reduction in proteinuria without a ≥ 25% reduction in eGFR) was not significantly associated with death (HR 0.96 [0.43-2.14], p=0.925), dialysis (HR 0.58 [0.16-2.06], p=0.406) or the composite endpoint (HR 0.98 [0.47-2.02], p=0.947).

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Univariable analyses using the ACC for cardiac organ response at 12 months were performed. Cardiac progression (increase in NT-proBNP of >30%) was a highly significant predictor of death (HR 5.32 [2.78-10.16], p<0.001) and the composite endpoint (HR 3.10 [1.61-5.96], p<0.001) but not dialysis, although demonstrated a trend to significance (HR 2.49 [0.91-6.78], p=0.075). Cardiac response (reduction in NT-proBNP of >30%) was a highly significant (protective) predictor of death (HR 0.24 [0.12-0.51], p<0.001) and the composite endpoint (HR 0.42 [0.22-0.80], p=0.009) but not dialysis (HR 0.54 [0.20-1.42], p=0.214).

Multivariable analyses for predictors of death, dialysis and the composite endpoint were performed including increase in NT-proBNP >30%, reduction in eGFR of ≥ 25%, and dFLC response of ≥90% at 12 months from baseline as categorical variables (Table 2b). An increase in NT-proBNP of >30% was a highly significant predictor of all three outcomes; death (HR 3.67 [1.79-7.53], p<0.001), dialysis (HR 2.85 [1.29-6.32], p=0.010) and the composite endpoint (3.05 [1.68-5.52], p<0.0001). Reduction in eGFR of ≥ 25% was not significantly associated with death (HR 1.23 [0.63-2.42], p=0.546) or the composite endpoint (HR 1.70 [0.96-3.05], p=0.070) but was associated with dialysis (HR 3.04 [1.28-7.25], p=0.012) and dFLC response of ≥90% was not significantly associated with any of the three outcomes. A landmark analysis was performed at 12 months comparing overall survival among patients who had a > 30% increase in NT-proBNP with patients who did not have a 30% increase in NT-proBNP at 12 months (Figure 1C).

## Discussion

Systemic AL amyloidosis is a multisystem disease with a heterogeneous clinical presentation and clinical course, as well as a widely varying prognosis. Symptoms, quality of life and patient survival are dependent upon the presence and severity of individual organ involvement

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and the hematologic response to chemotherapy, the mainstay of treatment for the disease. International amyloidosis consensus criteria (ACC) exist to define individual organ involvement by amyloid at the time of diagnosis as well as to define response of individual organs to chemotherapy. It is well established that presence and severity of cardiac amyloidosis at diagnosis is the main determinant of patient survival in AL amyloidosis,(Wechalekar, *et al* 2013) whilst presence and severity of renal dysfunction from amyloid determines the risk of progression to end stage renal disease (ESRD), a major determinant of quality of life. Systemic AL amyloidosis is a cause of cardiorenal syndrome, but there are no studies that have specifically looked at patient and renal survival in patients with systemic AL amyloidosis presenting with combined cardiac and renal amyloidotic dysfunction. Here we show for the first time that almost one third of patients diagnosed with systemic AL amyloidosis present with Type 5 cardiorenal syndrome, and that their outcomes are poor despite chemotherapy. Nearly two thirds of patients in the cardiorenal cohort died, among whom median time to death was only 5 months; median overall survival in this cohort by Kaplan Meier analysis was only ~18 months. In addition, 16% patients developed ESRD, the majority of whom reached dialysis within 12 months of diagnosis and median (range) time to the composite endpoint of either death or dialysis by KM analysis in the whole cohort was only 9.3 (5.5-13.1) months.

The data presented here are novel in that they concern three hard outcome measures, patient survival, dialysis, and the composite of either death or dialysis in the one third of patients with AL amyloidosis who present specifically with cardiorenal syndrome. At the time of diagnosis, the three disease-related variables that were highly significant independent predictors of all three outcomes were NT-proBNP concentration, Mayo Stage and eGFR. As previously and extensively reported,(Mahmood, *et al* 2014, Palladini, *et al* 2012, Pinney, *et al* 2011) the clonal response to chemotherapy within 12 months of diagnosis was also predictive of both patient and renal survival.

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Interestingly, an increase or reduction in proteinuria at 12 months, both of which are included in the ACC renal response criteria, did not predict death, dialysis, or the composite endpoint. Additionally, an 'early' renal response (at 6 months from baseline) was not significantly associated with any of the three outcome measures. A reduction of eGFR of $\geq 25\%$ at 6 months was predictive of dialysis and the composite endpoint in keeping with previous findings by Palladini and colleagues,(Palladini*, et al* 2014) whilst an increase in NT-proBNP of >30% at 12 months was predictive of all three outcome measures. This highlights for the first time the strong association between both baseline NT-proBNP concentration, its increase at 12 months (ACC cardiac progression) and each of the three hard outcome measures of death, dialysis and the composite endpoint. Furthermore, whilst renal stage (eGFR <50ml/min and proteinuria of >5g/24hr) at baseline was highly predictive of renal survival alone; proteinuria, both at baseline and its change at 6 and 12 months was not predictive of any of the three outcomes in this cohort of patients, possibly due to the high early 'event' rate for both death and dialysis.

An increase or reduction in proteinuria in the absence of worsening GFR has previously been shown to be predictive of renal survival in patients with isolated renal AL amyloidosis.(Pinney*, et al* 2011) The pathophysiology of reduction in proteinuria in AL amyloidosis is thought to relate to amyloid regression, or perhaps absence of ongoing amyloid accumulation, which is dependent upon adequate suppression of the underlying clonal disorder.(Gillmore*, et al* 2001) However, in clinical practice, a significant improvement in proteinuria rarely occurs before six months to one year; it may be therefore, that in this distinct cohort of patients with cardiorenal AL amyloidosis, the high early death, and to a lesser degree dialysis rates, preclude the use of proteinuria as a prognostic biomarker within the first year of diagnosis.

Based upon our study, we aimed to risk stratify patients with cardiorenal syndrome; at baseline, based upon eGFR and NT-proBNP (Table 3) and thereafter, based upon change in biomarkers within 12 months from diagnosis (Figure 2). At baseline, low risk was defined as an NT-proBNP of ≤8500ng/L and eGFR ≥ 30 ml/min/1.73 $m^2$, intermediate risk as either NT-proBNP of >8500ng/L or eGFR <30 ml/min/1.73 $m^2$ and high risk as NT-proBNP of >8500ng/L and eGFR <30 ml/min/1.73 $m^2$ at baseline. High risk compared to low risk patients had a substantially increased risk of death (HR 3.12 [2.18-4.64], p<0.001), dialysis (HR 10.34 [4.66-22.91], p<0.001) and the composite endpoint (HR 4.76 [3.24-7.00], p<0.001) (Table 3). There was a significant trend across the 3 categories of cardiorenal risk stage for all three outcomes (p≤0.007). In all patients with systemic AL amyloidosis and cardiorenal syndrome, the outcome of dialysis was predominantly dictated by ≥ 25% reduction in eGFR at 6 months, but at 12 months an increase in NT-proBNP of >30% was the most significant predictor of death, dialysis and the composite endpoint (Figure 2).

Limitations of our study include the relatively low incidence of dialysis in this cohort of patients. A total of 50 patients required dialysis of whom 50% were on dialysis by 6 months. Hemodialysis is the acute RRT therapy of choice in the UK and it may be that a small number of patients were deemed unsuitable to receive hemodialysis due to concurrent advanced cardiac amyloidosis, autonomic nerve dysfunction and associated hypotension as well as patient choice. Due to the nature of our referral centre, these details were not available to us. Nonetheless, the authors feel that this 'real world' data regarding the specific cohort of patients with systemic AL amyloidosis who have type 5 cardiorenal syndrome is key in guiding clinicians who counsel such patients about their treatment and outcomes within the first year of diagnosis.

In summary, approximately one third of patients with systemic AL amyloidosis have cardiorenal syndrome at the time of diagnosis. Despite the complex relationship between NT-

proBNP, renal excretory function, and cardiac function, our data indicates that hard outcome measures in patients with systemic AL amyloidosis and type 5 cardiorenal syndrome are predominantly dictated by both baseline NT-proBNP and its change at 12 months. The key renal biomarker in this cohort is eGFR and not proteinuria. The initial aim of therapy, be it chemotherapy to prevent ongoing accumulation of amyloid and progressive amyloidotic organ dysfunction or symptomatic management of fluid balance, should be to prevent loss of $\geq 25\%$ of baseline eGFR within 6 months and prevent an increase in NT-proBNP of $>30\%$ within 12 months of diagnosis. Our data suggest that, irrespective of changes in proteinuria, and to some degree, GFR, changes in NT-proBNP (ACC cardiac progression) may be the most important independent predictor of death or a requirement for renal replacement therapy in such patients. Further work, possibly including novel methods of tracking end organ response, is needed to refine the current amyloid consensus criteria in order to reflect the needs of patients with multi-system disease from AL amyloidosis.

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## Author Contributions

TR and JDG conceived the manuscript. HJL, MF, AM, SS, SM, CW, JP, DF, TL, TY and ADW were responsible for care and follow up of patients during the study. AP contributed to analysis and statistical results. PNH and JDG reviewed the final manuscript.

## Conflict of interest disclosures

None of the authors have any conflict of interest to declare

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# References

Comenzo, R.L., Reece, D., Palladini, G., Seldin, D., Sanchorawala, V., Landau, H., Falk, R., Wells, K., Solomon, A., Wechalekar, A., Zonder, J., Dispenzieri, A., Gertz, M., Streicher, H., Skinner, M., Kyle, R.A. & Merlini, G. (2012a) Consensus guidelines for the conduct and reporting of clinical trials in systemic light-chain (AL) amyloidosis. *Leukemia,* **26,** 2317-2325.

Comenzo, R.L., Reece, D., Palladini, G., Seldin, D., Sanchorawala, V., Landau, H., Falk, R., Wells, K., Solomon, A., Wechalekar, A., Zonder, J., Dispenzieri, A., Gertz, M., Streicher, H., Skinner, M., Kyle, R.A. & Merlini, G. (2012b) Consensus guidelines for the conduct and reporting of clinical trials in systemic light-chain amyloidosis. *Leukemia,* **26,** 2317-2325.

Dispenzieri, A., Zhang, L., Katzmann, J.A., Snyder, M., Blood, E., Degoey, R., Henderson, K., Kyle, R.A., Oken, M.M., Bradwell, A.R. & Greipp, P.R. (2008) Appraisal of immunoglobulin free light chain as a marker of response. *Blood,* **111,** 4908-4915.

Fontana, M., Chung, R., Hawkins, P.N. & Moon, J.C. (2015) Cardiovascular magnetic resonance for amyloidosis. *Heart Fail Rev,* **20,** 133-144.

Gertz, M.A., Comenzo, R., Falk, R.H., Fermand, J.P., Hazenberg, B.P., Hawkins, P.N., Merlini, G., Moreau, P., Ronco, P., Sanchorawala, V., Sezer, O., Solomon, A. & Grateau, G. (2005) 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. *Am. J. Hematol.,* **79,** 319-328.

Gillmore, J.D., Lovat, L.B., Persey, M.R., Pepys, M.B. & Hawkins, P.N. (2001) Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein. *Lancet,* **358,** 24-29.

Hawkins, P.N., Lavender, J.P. & Pepys, M.B. (1990) Evaluation of systemic amyloidosis by

    scintigraphy with ^{123}I-labeled serum amyloid P component. *N. Engl. J. Med.,* **323,**

    508-513.

Hutchison, C.A., Harding, S., Hewins, P., Mead, G.P., Townsend, J., Bradwell, A.R. &

    Cockwell, P. (2008) Quantitative assessment of serum and urinary polyclonal free

    light chains in patients with chronic kidney disease. *Clin. J. Am. Soc. Nephrol.,* **3,**

    1684-1690.

Kyle, R.A., Greipp, P.R. & O'Fallon, W.M. (1986) Primary systemic amyloidosis:

    multivariate analysis for prognostic factors in 168 cases. *Blood,* **68,** 220-224.

Maceira, A.M., Joshi, J., Prasad, S.K., Moon, J.C., Perugini, E., Harding, I., Sheppard, M.N.,

    Poole-Wilson, P.A., Hawkins, P.N. & Pennell, D.J. (2005) Cardiovascular magnetic

    resonance in cardiac amyloidosis. *Circulation,* **111,** 186-193.

Mahmood, S., Palladini, G., Sanchorawala, V. & Wechalekar, A. (2014) Update on treatment

    of light chain amyloidosis. *Haematologica,* **99,** 209-221.

Palladini, G., Campana, C., Klersy, C., Balduini, A., Vadacca, G., Perfetti, V., Perlini, S.,

    Obici, L., Ascari, E., d'Eril, G.M., Moratti, R. & Merlini, G. (2003) Serum N-terminal

    pro-brain natriuretic peptide is a sensitive marker of myocardial dysfunction in AL

    amyloidosis. *Circulation,* **107,** 2440-2445.

Palladini, G., Dispenzieri, A., Gertz, M.A., Kumar, S., Wechalekar, A., Hawkins, P.N.,

    Schonland, S., Hegenbart, U., Comenzo, R., Kastritis, E., Dimopoulos, M.A., Jaccard,

    A., Klersy, C. & Merlini, G. (2012) 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.,* **30,** 4541-4549.

Palladini, G., Hegenbart, U., Milani, P., Kimmich, C., Foli, A., Ho, A.D., Vidus Rosin, M.,

    Albertini, R., Moratti, R., Merlini, G. & Schonland, S. (2014) A staging system for

renal outcome and early markers of renal response to chemotherapy in AL amyloidosis. *Blood,* **124,** 2325-2332.

Pepys, M.B. (2006) Amyloidosis. *Annu. Rev. Med.,* **57,** 223-241.

Pinney, J.H., Lachmann, H.J., Bansi, L., Wechalekar, A.D., Gilbertson, J.A., Rowczenio, D., Sattianayagam, P.T., Gibbs, S.D.J., Orlandi, E., Wassef, N.L., Bradwell, A.R., Hawkins, P.N. & Gillmore, J.D. (2011) Outcome in Renal AL Amyloidosis following Chemotherapy. *Journal of Clinical Oncology,* **29,** 674-681.

Rezk, T., Lachmann, H.J., Fontana, M., Sachchithanantham, S., Mahmood, S., Petrie, A., Whelan, C.J., Pinney, J.H., Foard, D., Lane, T., Youngstein, T., Wechalekar, A.D., Bass, P., Hawkins, P.N. & Gillmore, J.D. (2017) Prolonged renal survival in light chain amyloidosis: speed and magnitude of light chain reduction is the crucial factor. *Kidney Int,* **92,** 1476-1483.

Ronco, C., Haapio, M., House, A.A., Anavekar, N. & Bellomo, R. (2008) Cardiorenal syndrome. *J Am Coll Cardiol,* **52,** 1527-1539.

Soni, S.S., Ronco, C., Pophale, R., Bhansali, A.S., Nagarik, A.P., Barnela, S.R., Saboo, S.S. & Raman, A. (2012) Cardio-renal syndrome type 5: epidemiology, pathophysiology, and treatment. *Semin Nephrol,* **32,** 49-56.

Wechalekar, A.D., Schonland, S.O., Kastritis, E., Gillmore, J.D., Dimopoulos, M.A., Lane, T., Foli, A., Foard, D., Milani, P., Rannigan, L., Hegenbart, U., Hawkins, P.N., Merlini, G. & Palladini, G. (2013) A European collaborative study of treatment outcomes in 346 patients with cardiac stage III AL amyloidosis. *Blood,* **121,** 3420-3427.

## Figure Legends

**Figure 1.** Landmark analyses at 6 (A & B) and 12 (C) months from diagnosis. A) Patients who had an increase in NT-proBNP of >30% at 6 months had a significantly shortened overall survival compared to those who had a stable (or reduced) NT-proBNP at the same time point (p=0.006). B) Patients who had a reduction in eGFR of ≥ 25% at 6 months had a significantly shortened renal survival compared to those who had a stable (or improved) eGFR at the same time point (p=0.003). C) Patients who had an increase in NT-proBNP of >30% at 12 months had a significantly shortened overall survival compared to those who had a stable (or reduced) NT-proBNP at the same time point (p<0.0001)

**Figure 2.** Risk stratification of patients with systemic AL amyloidosis and cardiorenal syndrome based upon NT-proBNP and eGFR at different timepoints within the course of the first year after diagnosis.