Title: Immunoparesis defined by heavy+light chain suppression is a novel marker of **long-term outcomes in cardiac AL amyloidosis**

Running title: HLC suppression in AL amyloidosis

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SUMMARY

Cardiac involvement and presenting dFLC (difference between involved and uninvolved free light chains) are independent predictors of outcome in systemic AL amyloidosis. These markers have limited prognostic utility in patients surviving the initial months following diagnosis. Here we assessed immunoparesis, as determined by novel heavy+light chain (HLC) immunoassays, as a prognostic marker for survival in AL amyloidosis. HLC measurements identified immunoparesis of at least one immunoglobulin (Ig) isotype in 145(85%) patients; and severe immunoparesis (≥2 Ig isotypes suppressed by >50% below normal levels) in 29(17%) patients. Median overall survival (OS) on intention to treat (ITT) analysis was 26.2 months. In the ITT cohort, dFLC >180mg/L was associated with shorter OS (p=0.05); whereas HLC immunoparesis was not prognostic. On a landmark analysis of 127 patients alive at 6 months, presenting dFLC was not prognostic for OS (p=0.33) and severe HLC immunoparesis trended towards poorer survival (20.2 vs. 42.8 months; p=0.09). In the subset of patients with cardiac involvement, severe HLC immunoparesis conferred very poor outcome (median OS 8.8 vs. 29.9 months, p=0.007). In conclusion severe HLC immunoparesis is an independent marker of long-term poor prognosis in AL patients with cardiac involvement. The pathophysiological significance of this observation needs further study.

Keywords: Hevylite, immunoparesis, amyloid, cardiac, Freelite

INTRODUCTION

Systemic light chain amyloidosis (AL amyloidosis) is a rare disorder of misfolded monoclonal immunoglobulin free light chains (FLC), which result in insoluble fibrils deposition in organs causing progressive organ dysfunction. The amyloidogenic FLC are products of an underlying B cell clonal disorder (usually a plasma cell clone) and current therapeutic strategies are aimed at targeting such clones to reduce the levels of circulating clonal FLC. The prognosis in a patient with systemic AL amyloidosis depends on the extent of end organ damage – particularly cardiac impairment. Additionally, the level of circulating FLC also has independent prognostic significance (Comenzo*, et al* 2012, Dispenzieri*, et al* 2009, Kumar*, et al* 2012, Palladini*, et al* 2012); as has achieving a deep biochemical response to therapy. A substantial proportion of patients (up to 30% in some series) succumb prematurely to disease related complications, primarily due to advanced cardiac involvement (Dispenzieri*, et al* 2004). Hence the prognostic factors in the early survivors (i.e. outcomes on landmark analyses) are very different from those of 'intention to treat analysis' at baseline. The value of current amyloidosis staging systems in assessing longer term prognosis of patients surviving past the initial few months remains unclear and appears to be limited.

In addition to free light chains, monoclonal intact immunoglobulins (M-Igs) are also expressed in about 50-75% of patients with AL amyloidosis, although only in about 25% of patients the levels are measurable for monitoring purposes (Lachmann*, et al* 2003). The prognostic value of an intact M-Ig in systemic AL amyloidosis is unclear, with some series reporting poorer outcomes for patients expressing intact immunoglobulins (Kumar*, et al* 2010, Kumar*, et al* 2011). Alternatively and similar to other plasma cell dyscrasias, systemic immunoparesis has recently been associated with poorer outcomes in AL amyloidosis patients (Muchtar*, et al* 2016b).

Novel serum assays allow quantification of Ig'κ and Ig'λ heavy+light chains (HLC) from which Ig'κ / Ig'λ HLC ratios can be derived. This may give an indication of clonality and HLC immunoassays appear to be sensitive for identifying and quantifying levels of M-Ig in plasma cell dyscrasias (Bijzet*, et al* 2012, Ludwig*, et al* 2013, Schonland*, et al* 2012). The particular

advantage of HLC immunoassays over traditional methods of immunoglobulin measurements is that it allows the quantification of the uninvolved (non-clonal) member of the pair as well as of the other immunoglobulin classes (e.g. in an IgGκ monoclonal protein expressing patient, levels of IgGλ, IgAκ, IgAλ, IgMκ and IgMλ may be measured) –thus providing an accurate measurement of isotype and non-isotype specific immunoglobulin values as well as pair immunosuppression.

There are few studies evaluating the role of HLC suppression for prognostication in plasma cell dyscrasias. In myeloma, HLC suppression appears to predict for poorer outcomes (Avet-Loiseau*, et al* 2011, Harutyunyan*, et al* 2016, Ludwig*, et al* 2016). Here we report the significance of immunoparesis as determined by HLC suppression in a population of newly diagnosed patients with systemic AL amyloidosis.

METHODS

The study included unselected patients with AL amyloidosis seen at the National Amyloidosis Centre in London between 2003-2008, with serum samples collected at the time of presentation, prior to any therapy, and stored at -80°C. A total of 170 patients with systemic AL amyloidosis were included. There was no blinding in the study. Diagnosis of amyloidosis was confirmed in all cases with a tissue biopsy demonstrating characteristic birefringence on Congo red staining. Typing of AL amyloidosis was confirmed by immunohistochemical staining with appropriate antibodies and by exclusion of hereditary amyloidosis, where necessary, by genetic sequencing of the genes implicated. Patients fulfilling criteria for symptomatic myeloma were excluded. All patients underwent systematic review at presentation and detailed follow up assessments at 6 monthly intervals or as clinically indicated. Assessment included clinical examination, detailed blood and urine analysis (including assessment of serum and urine monoclonal immunoglobulin and serum free light chains), serial ^{123}I labelled serum amyloid P component (SAP) scintigraphy to assess whole body amyloid load, electrocardiogram (ECG) and echocardiogram. Organ involvement was defined according to the international amyloidosis consensus criteria (Gertz*, et al* 2004a). Patients were treated with chemotherapy regimens used to treat myeloma during the study period. Treatment information was available for 109 (66 %) of the patients. Treatment regimen included those based on thalidomide (37%), melphalan (13%), cyclophosphamide (4%) and VAD (2%).

Serum samples were tested for FLC concentrations (κ sFLC and λ sFLC) using Freelite® assays (The Binding Site Group Ltd, UK), HLC concentrations (IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ) using Hevylite® assays (The Binding Site Group Ltd, UK) and total immunoglobulins (IgG, IgA and IgM) on a BN™II System nephelometer (Siemens, Germany). Serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE) (Sebia, France) were carried out using standard laboratory procedures. Immunoparesis was defined either by total immunoglobulin (Ig) measurements as the concentration of any Ig class below the lower limit of normal (i.e. IgG<6 g/L, IgA<0.8 g/L, IgM<0.5 g/L; total Ig suppression), or by HLC immunoassays as levels of any IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and/or IgMλ below the lower

limit of their respective reference range (HLC suppression; Supplementary Table 1). Moderate immunoparesis was defined as at least two Ig isotypes suppressed below the lower limit of normal, and severe immunoparesis was defined as levels of two or more isotypes suppressed by 50% or greater below the lower limit of normal.

Survival studies were performed on 163 patients with available follow-up data (median follow up 35 months (2.4 – 85.3 months). Cardiac involvement for survival studies was defined as above and/or NT-proBNP >332 ng/L. There were 108 deaths of which 8 were due to infection and 89 due to amyloidosis. Differences in overall survival (OS) between patient groups were analysed using Kaplan-Meier survival curves with the log rank test used to indicate significance. The association of variables with OS was performed with Cox proportional hazard model. A landmark analysis was carried out in patients surviving 6 months from study entry. p values were two-tailed with a significance level of 0.05. Statistical analyses were performed using SPSS v21 (IBM, Chicago, USA). Statistical differences for categorical values were calculated using the chi-square (χ^2) test. Survival graphs were generated using GraphPad/Prism 5 software.

The study was carried out in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki, and was approved by the institutional review board. All participating patients provided written informed consent.

RESULTS

Baseline characteristics including demographics, clinical features and serum biomarkers for 170 AL amyloidosis patients are presented in Table 1 – 73% and 63% respectively had cardiac and renal involvement. None of the patients had symptomatic myeloma but 21% had greater than 10 % bone marrow plasma cell infiltration. **The association of baseline variables with HLC and total Ig immunoparesis is presented on Table 1; and on supplementary Tables 2 and 3 for moderate and severe immunoparesis, respectively. Our analyses mainly revealed an association between any degree of immunoparesis and an abnormal serum κ/λ FLC ratio.**

HLC measurements identified immunosuppression of at least one of any IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ in 145/170 (85%) patients (Table 2). Specifically, IgGk levels were suppressed in 108 (64%) patients, IgGλ in 75 (44%), IgAk in 56 (33%), IgAλ in 42 (25%), IgMk in 79 (47%) and IgMλ in 70 (41%). In addition 80/170 (47%) patients presented with moderate HLC immunoparesis. Severe immunoparesis was identified by HLC measurements in 29/170 (17%) patients (Table 2).

Total Ig measurements identified immunoparesis of at least one of any IgG, IgA and IgM in 115/166 (69%) patients; all of which were identified by HLC suppression. 56/166 (34%) patients had two or more immunoglobulins below normal levels, and in 18/166 (11%) patients immunoparesis was severe (Table 2).

We assessed the impact of immunoparesis on survival outcomes. There were 108 deaths. Infection was reported as a cause of death in 8 cases. Other reported causes included: acute leukaemia – 1, gastrointestinal bleeding – 2, myocardial infarction – 1, renal failure - 3, treatment related - 4 and all remaining cases – progressive amyloidosis or amyloidosis. Median survival for this cohort was 26.2 months. In the intent to treat (ITT) cohort, presence of **more than 1 organ involved**, cardiac involvement, abnormal NT-proBNP and high monoclonal FLC levels were factors adversely affecting survival by univariate analysis, **and trended towards significance in multivariate**; whereas the presence of an M-protein, an abnormal HLC ratio, and immunoparesis by either method had no significant impact on

survival (Table 3). Median OS for patients with dFLC levels above or below 180 mg/L was 14.8 vs. 43.1 months, respectively (Hazard Ratio (HR) (95%CI): 1.6 (1.0-2.4); p=0.05), whereas median survival for patients with severe HLC immunoparesis was 14.8 months compared to 28.0 months for all other patients (HR: 1.4 (0.9-2.3); p=0.16) (Figure 1A-C). We further assessed the survival characteristics of 121 patients who presented with cardiac involvement. **In multivariate analysis, more than 1 organ affected,** dFLC >180 mg/L and severe HLC immunoparesis were associated with poorer survival (Table 3). Median OS for this cardiac population was 14.8 months. Median OS for dFLC >180 mg/L vs. dFLC <180 mg/L was 12.6 and 35.1 months, respectively (HR: 1.7 (1.0-2.9); p=0.04); and severe HLC immnoparesis vs. all other patients was 8.8 and 21 months respectively (HR: 1.7 (1.0-2.9); p=0.06) (Figure 1D-F). Severe immunoparesis as determined by total Ig measurements had no impact on prognosis in cardiac patients (p=0.81; Table 3).

We carried out a landmark analysis of 127 patients who survived for at least 6 months (Table 3 and Figure 2). Median survival for this population was 40.9 months. **Cox regression and Kaplan-Meier analysis revealed organ involvement and dFLC levels no longer associated with outcome in this cohort. There was a suggestion that patients with severe HLC suppression had a trend towards poorer survival (p=0.09). Indeed, in patients with cardiac involvement, in the 6-month landmark analysis, severe HLC suppression (HR: 2.4 (1.2-4.6); p=0.009) was the only variable associated with shorter overall survival.** A landmark analysis at nine and twelve months of patients with cardiac involvement and HLC suppression also demonstrated association with survival, with median OS of 58.5 and 63.7 months for those with mild/no HLC suppression at nine and twelve months, respectively, compared to 28.4 months for those with severe HLC suppression (p=0.111 and p=0.059 respectively; supplementary Figure 1).

A tentative survival model including dFLC >180mg/L and severe HLC suppression as risk factors in patients with cardiac involvement stratified the population into three categories with none (n=49), one (n=59) and two (n=13) risk factors and median survival times of 35.1, 12.7 and 8.8 months, respectively (p=0.023) (Figure 3).

DISCUSSION

The impact of immunoparesis on outcomes in plasma cell dyscrasias has been studied and debated for many years, but only recently has been specifically assessed in AL amyloidosis. Here we report that immunoparesis is common in systemic AL amyloidosis by both standard nephelometric immunoglobulin measurements and as determined by HLC immunoassays. Our main finding indicates that severe immunoparesis measured by HLC immunoassay, but not by total Ig measurements, is a marker of poor prognosis particularly in patients with cardiac amyloidosis who survive the first few months after therapy.

Impact of M-Ig on outcomes in AL amyloidosis is unclear. The Mayo group reported that response to treatment as determined by FLC but not by intact M-Ig impacted survival in AL, both in chemotherapy and transplant settings (Kumar*, et al* 2010, Kumar*, et al* 2011)– data which was confirmed by a collaborative study defining response criteria in AL amyloidosis (Palladini*, et al* 2012). The current cohort confirms these findings; where the presence of an M-Ig by IFE had no prognostic value. Traditional electrophoretic methods lack sensitivity for detecting M-Ig and cannot accurately quantify the low levels typically encountered in patients with systemic AL amyloidosis (Harding*, et al* 2010, Lachmann*, et al* 2003). HLC immunoassays have a greater sensitivity for detection of M-Ig's and may aid the monitoring and prognostication of monoclonal gammopathies (Avet-Loiseau*, et al* 2009, Katzmann*, et al* 2013, Ludwig*, et al* 2013).

In patients with multiple myeloma, extreme abnormality of HLC ratio at presentation (<0.01 or >200) was associated with shorter time to disease progression in IFM 2005-01MM trial patients (Bradwell*, et al* 2013). It also associates with shorter progression free survival and overall survival (Bradwell*, et al* 2013, Koulieris*, et al* 2012). The suppression of the uninvolved immunoglobulin of the same heavy chain isotype appears to be a major contributor to this prognostic impact; it also predicts for shorter time to progression in MGUS (Katzmann*, et al* 2013).

Unlike myeloma, extreme HLC ratios are rarely seen in AL amyloidosis. By contrast, immunoparesis is nearly universal in myeloma and seen in a proportion of patients with monoclonal gammopathy of undetermined significance (MGUS) (Katzmann*, et al* 2013). However, systemic immunoparesis as determined by total Ig measurements remains an inconsistent risk factor both in MGUS and MM (Cesana*, et al* 2002, Kastritis*, et al* 2014, Katzmann*, et al* 2013, Turesson*, et al* 2014). We found a greater incidence of HLC suppression (85%) over total Ig immunoparesis (69%) in AL amyloidosis. Differences may partly be due to the ability of HLC immunoassays to separately identify kappa and lambda isotypes of each Ig class, unlike total Ig measurements. An increased frequency of HLC suppression was also reported in a study in MGUS, in which 27% and 11% of 999 patients displayed immunoparesis as determined by HLC and total Ig measurements, respectively (Katzmann*, et al* 2013). We found no correlation between immunoglobulin suppression and NT-proBNP or monoclonal FLC levels, indicating that polyclonal immunoglobulin levels do not associate with other risk factors or stage of disease in AL amyloidosis (Sachchithanantham*, et al* 2014).

Baseline level of dFLC was prognostic in this cohort, as reported previously from other studies in AL amyloidosis (Goodman*, et al* 2005, Palladini*, et al* 2012)– the absolute value of the amyloidogenic protein was directly linked with prognosis. However, in the landmark analysis of survivors at six months, the dFLC lacked prognostic power. The biggest challenge in AL amyloidosis is early deaths due to disease related complications. Patients surviving beyond six months have demonstrated resilience of organ function and have much better outcomes (Wechalekar*, et al* 2013). Baseline biomarkers do not have the same prognostic impact on the six month survivors (Wechalekar*, et al* 2012). The impact of baseline dFLC on survivors has (or indeed the lack of prognostic impact of baseline dFLC on survivors as seen here), to the best of our knowledge, never been previously reported.

The striking observation in this series was the impact of severe HLC immunoparesis specifically on patients with cardiac involvement in a landmark analysis. Cardiac involvement remains the major prognostic determinant in AL patients

and risk-adapted strategies have been suggested whereby the aggressiveness of the treatment regimen is inversely proportional to the extent of cardiac disease, as determined by cardiac biomarkers (Gertz*, et al* 2004b, Palladini and Merlini 2009, Palladini*, et al* 2009). There is a high likelihood that the gentler regimes are not able to achieve a deep clonal response and hence are less efficient in allowing polyclonal immunoglobulin recovery – a feature associated with improved outcomes in myeloma (Gonzalez-Calle*, et al* 2017, Harutyunyan*, et al* 2016, Ludwig*, et al* 2016, Tovar*, et al* 2012); or indeed whether there is higher risk of opportunistic infections or related complications contributing to mortality in a compromised cardiovascular patient. In this context it would be relevant to assess whether novel therapies, including bortezomib and immunomodulatory based regimens, may overcome the adverse effect of HLC immunoparesis in cardiac patients (Muchtar*, et al* 2016b) through more efficient targeting of the clone and a boost to polyclonal immune activation (Zamarin*, et al* 2013).

By contrast, immunoparesis by total Ig measurement (even severe immunoparesis), had no impact on prognosis in this study. A recent report from the Mayo group showed that classical immunoparesis is prognostic in AL amyloidosis (Muchtar*, et al* 2016a, Muchtar*, et al* 2016b). The same group previously reported no association between immunoparesis and outcome in a small cohort of 41 AL amyloidosis patients (Muchtar*, et al* 2016c), intimating that sample size may account in part for the discrepant results between studies; and that the sensitivity of Hevylite assays may be potentially greater in detecting this effect – the Mayo study did not include HLC assessments.

The impact of immunoparesis in AL patients with renal involvement requires careful interpretation, as patients with nephrotic syndrome lose IgG into the urine as a result of increased glomerular permeability. In these cases, immunoparesis may reflect IgG loss rather than plasma cell suppression (Kaysen and al Bander 1990). Similar to what has been described in patients with moderate immunoparesis in other series (Muchtar*, et al* 2016a) this may explain why moderate HLC suppression did not associate with outcome in our patients.

The mechanism of suppression of normal immunoglobulin components in plasma cell dyscrasias in AL remains poorly understood but is likely to be directly related to the characteristics of the bone marrow plasma cell clone. The group from Salamanca had reported that the presence of <5% normal plasma cells defined by multiparameter flow cytometry conferred a poor prognosis (Paiva*, et al* 2011). Similarly, we reported that presence of >10% normal plasma cells in the marrow by flow cytometry, irrespective of absolute plasma cell percentage by morphology (also a prognostic factor as reported by the Mayo group), predicted for better outcomes (Sachachtanatham*, et al* 2014). Since normal immunoglobulin production is from persisting normal plasma cells in the bone marrow, the suppression of normal immunoglobulins as determined by HLC possibly represents the serum manifestation of this phenomenon. We are planning a further study of HLC correlating serum findings with baseline bone marrow assessments to confirm these observations.

Whilst this study shows the important prognostic impact of HLC immunoparesis on outcomes in AL amyloidosis, the reason for this prognostic impact is far more challenging to dissect out. Infections and worsening heart failure are the commonest causes of serious adverse events in patients with AL amyloidosis undergoing chemotherapy (Wechalekar*, et al* 2014). Drugs commonly used in treatment of AL amyloidosis such as dexamethasone or cyclophosphamide are excellent immunosuppressive agents, and are likely to eliminate normal plasma cells in addition to achieving the desired impact of clonal eradication. Since HLC immunoparesis of the non-clonal uninvolved immunoglobulin is most likely to be directly linked to greater suppression/depletion of normal plasma cells, it is tempting to speculate that such patients with severe HLC immunoparesis will have worsening immunodeficiency during treatment; which could tip these patients into a longer term state of immunodeficiency. The prognostic impact of HLC immunosuppression is greatest soon after completing therapy (i.e. in the six month landmark analysis) compared to patients alive at 9 or 12 months, suggesting that there might be immune recovery in the survivors; thereby mitigating the prognostic impact of immunoparesis. This immunoparesis may not only predispose to infective complication but may be a marker for poorer immune surveillance which may impact the longer term outcomes

in plasma cell dyscrasia. An alternative, or even concurrent, reason may be that the suppression of normal plasma cells is direct marker for the aggressiveness of the plasma cell clone as impacting on treatment responsiveness and possible persistence of minimal residual disease (MRD) with the attendant longer term consequences. We recently reported the persistence of MRD in AL amyloidosis patients in a serological CR (Coyne*, et al* 2015), highlighting the difficulty of eradicating even a small clone. We hope that an ongoing serial study of HLC monitoring in AL patients during and after treatment may address some of these questions.

This study has limitations and these observations need to be validated in a larger patient population **treated with novel agents**. The availability of baseline sera stored at the requisite temperatures dictated patient inclusion in this study. Baseline troponin measurement was not part of standard patient assessment at the UK NAC at time of this study and hence we are unable to present this data. We plan to expand this study to include HLC as part of baseline assessments in patients included in our ongoing observational study (ALCHemy) to validate these findings in a series of prospectively observed patient cohort. Similarly, the retrospective nature of the data limits the ability to assess cause of death and impact, if any, of infections due to immunoparesis; particularly worsened after chemotherapy.

In summary, immunoparesis as defined by HLC suppression and total Ig measurements is a relatively common occurrence in AL amyloidosis. Severe HLC immunoparesis appears to be a marker of poor prognosis in patients with cardiac amyloidosis and is a particularly powerful marker in survivors beyond the first six months from diagnosis. The clinical benefit of routine HLC measurements in patients with AL amyloidosis warrants further exploration in larger longitudinal studies.

Authors' contributions

SS and ADW designed the study. SS, SAM, HJL, JDG, PNH and ADW recruited patients and samples. SS, ADW, OB, AA and SH analysed data and wrote the manuscript. All authors discussed the data and critically appraised the manuscript.

Conflicts of interest

SS, SAM, HJL, JDG, PNH and ADW declare no conflicts of interest. OB, AA and SH are Binding Site employees.

Figure legends

Figure 1. Survival outcomes in intention-to-treat (ITT) cohort. (A) Median overall survival (OS) for the whole cohort (n=163) and (B) for patients stratified by baseline dFLC >180mg/L and (C) severe HLC suppression (≥50% suppression in ≥2 Ig isotypes). (D) Median OS for patients with cardiac involvement at diagnosis (n=121) and (E) stratified by baseline dFLC >180mg/L and (F) severe HLC suppression. Number of patients (deaths) for each arm is shown.

Figure 2. Survival outcomes in 6-month landmark analysis. (A) Median overall survival (OS) for patients alive at six months (n=127) and (B) based on baseline dFLC >180mg/L and (C) severe HLC suppression (≥50% suppression in ≥2 Ig isotypes). (D) Median OS in the 6-month landmark analysis for patients with cardiac involvement (n=89) and (E) stratified by baseline dFLC >180mg/L and (F) severe HLC suppression. Number of patients (deaths) for each arm is shown.

Figure 3. OS survival for patients with cardiac involvement stratified by baseline risk factors (dFLC >180mg/L and severe HLC suppression). In 121 patients with cardiac involvement (cardiac disease and/or NT-pro BNP > 332ng/L) presence of none (blue line), 1 (green line) or 2 (red line) risk factors identified three groups with median survival times of 35.1, 12.7 and 8.8 months, respectively (p=0.02). Number of patients (deaths) for each arm is shown.

Supplementary Figure 1. Survival outcomes in 9- and 12-month landmark analysis. Median OS in the (A) 9-month (n=77) and (B) 12-month (n=68) landmark analysis for patients with cardiac involvement and severe HLC suppression (≥50% suppression in ≥2 Ig isotypes). Number of patients (deaths) for each arm is shown.

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Figure 1

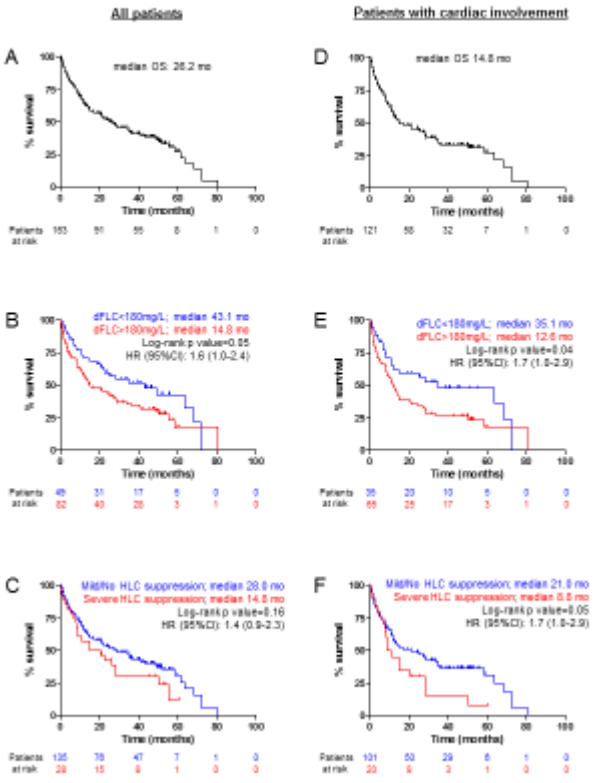
All patients
Patients with cardiac involvement
A
median OS: 26.2 mo
D
median OS 14.8 mo
% survival
Time (months)
Patients at risk
B
dFLC<180mg/L; median 43.1 mo
dFLC>180mg/L; median 14.8 mo
Log-rank p value=0.05
HR (95%CI): 1.6 (1.0-2.4)
E
dFLC<188mg/L; median 35.1 mo
dFLC>188mg/L; median 12.6 mo
Log-rank p value=0.04
HR (95%CI): 1.7 (1.0-2.9)
C
Mild/No HLC suppression; median 28.8 mo
Severe HLC suppression; median 14.8 mo
Log-rank p value=0.16
HR (95%CI): 1.4 (0.9-2.3)
F
Mild/No HLC suppression; median 21.8 mo
Severe HLC suppression; median 8.8 mo
Log-rank p value=0.05
HR (95%CI): 1.7 (1.0-2.9)

Figure 2

6-month landmark

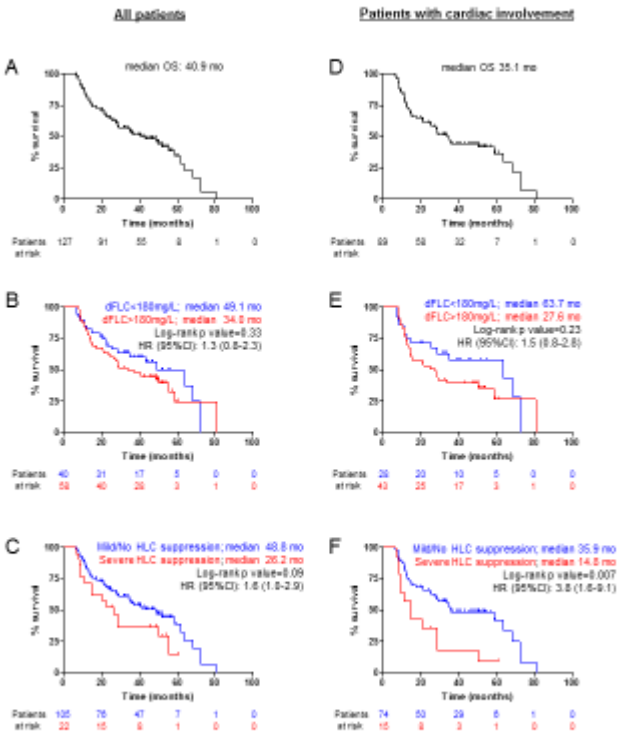

Figure 3

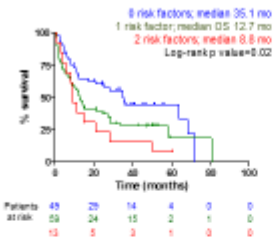
0 risk factors; median 35.1 mo
1 risk factor; median OS 12.7 mo
2 risk factors; median 8.8 mo
Log-rank p value=0.02
% survival
Time (months)
Patients at risk

Table 1. Baseline characteristics of patients and association with immunoparesis

	All patients	HLC immunoparesis			total Ig immunoparesis[4]		
Median (range) or n (%)	n=170	yes (n=145)	no (n=25)	p	yes (n=115)	no (n=51)	p
Age years	68 (34 - 85)	67 (34 - 85)	69 (52 - 82)	0.12	67 (34 - 85)	68 (50 - 85)	0.28
Age>65	96 (56)	77 (53)	19 (76)	0.03	61 (53)	31 (61)	0.35
Male	104 (61)	92 (63)	12 (48)	0.14	73 (64)	29 (57)	0.42
Cardiac involvement[1]	124 (73)	106 (73)	18 (72)	0.91	86 (75)	35 (69)	0.41
Kidney involvement	104 (63)	91 (64)	13 (57)	0.51	72 (63)	31 (66)	0.69
Liver involvement	45 (27)	39 (27)	6 (27)	1.00	27 (24)	16 (34)	0.18
PNS involvement	5 (3)	5 (4)	0 (0)	0.36	3 (3)	2 (4)	0.59
GI tract involvement	16 (10)	15 (11)	1 (4)	0.33	12 (11)	4 (8)	0.64
>1 organ involved	66 (39)	58 (40)	8 (32)	0.45	44 (38)	21 (41)	0.28
NTproBNP ng/L	1894 (9 - 69999)	1852 (9 - 69999)	3011 (34 - 51410)	0.77	1776 (59 - 69999)	2576 (9 - 51410)	0.72
Creatinine mmol/L	96 (19-851)	96 (19 - 851)	111 (53 - 688)	0.08	93 (19 - 770)	104 (45 - 851)	0.15
24h proteinuria g	3.1 (0.1-15.6)	3.5 (0.1 - 14.9)	3.0 (0.1 - 15.6)	0.44	3.8 (0.1 - 14.9)	3.1 (0.1 - 15.6)	0.66
Albumin g/L	35 (12-52)	34 (12 - 52)	37 (22 - 46)	0.02	34 (12 - 52)	35 (17 - 48)	0.25
Alkaline phosphatase IU/L	103 (27-3891)	105 (35 - 3891)	98 (27 - 625)	0.39	105 (35 - 3891)	100 (27 - 1144)	0.52
Abnormal κ/λ sFLC ratio	134 (79)	119 (82)	15 (60)	0.01	99 (86)	31 (61)	<0.01
dFLC >180 mg/L	83/134 (62)	75/119 (63)	8/15 (53)	0.47	62/99 (63)	18/31 (58)	0.65
dFLC mg/L	237.9 (9.9 - 5026.8)	263.3 (15.9 - 5027.0)	191.8 (9.9 - 4615.0)	0.18	263.3 (15.9 - 5027.0)	210.8 (9.9 - 4615.0)	0.57
Abnormal HLC ratio[2]	110 (65)	96 (66)	14 (56)	0.32	74 (64)	34 (68)	0.77
Intact M-Ig (by IFE)	87 (51)	78 (54)	9 (36)	0.10	57 (50)	28 (55)	0.53
Treatment categories[3]	n=109	yes (n=95)	no (n=14)		yes (n=78)	no (n=29)	
Thalidomide based	60 (55)	55 (58)	5 (36)		43 (55)	16 (55)	
Melphalan based	22 (20)	19 (20)	3 (21)		16 (21)	6 (21)	
Cyclophosphamide	8 (7)	5 (5)	3 (21)	0.15	5 (6)	3 (10)	0.75
VAD	4 (4)	4 (4)	0 (0)		4 (5)	0 (0)	
Other	15 (14)	12 (13)	3 (21)		10 (13)	4 (14)	

[1] *Determined by presence of cardiac amyloid deposits and/or NTproBNP>332ng/L;* [2] *9 patients had more than one Ig class with an abnormal HLC ratio;* [3] *n=109 patients with treatment data;* [4] *4 patients missed Ig data*

Table 2. Frequency of immunoparesis by method

Method	n	≥1 Ig suppressed n (%)	≥2 Ig suppressed (moderate suppression) n (%)	≥2 Ig suppressed >50% (severe suppression) n (%)
HLC suppression	170	145 (85)	80 (47)	29 (17)
Total Ig suppression	166*	115 (69)	56 (34)	18 (11)

		HLC (≥1 Ig suppressed)		
		No	Yes	Total
Total Ig (≥1 Ig suppressed)	No	25	26	51
	Yes	0	115	115
	Total	25	141	166

*4/170 patients missing total Ig measurements

Table 3. Cox regression analysis of risk factors for overall survival

	All patients				Patients with heart involvement and/or NT-proBNP>332ng/L			
	OS		6-month landmark OS		OS		6-month landmark OS	
	(n=163)		(n=127)		(n=121)		(n=89)	
	HR (CI)	p	HR (CI)	p	HR (CI)	p	HR (CI)	p
Univariate								
Heart involvement	2.2 (1.5-3.4)	**<0.01**	1.8 (1.1-3.0)	**0.02**	-	-	-	-
NT-proBNP >332 ng/L	2.3 (1.3-4.2)	**<0.01**	1.8 (0.9-3.6)	**0.08**	-	-	-	-
Age >65	1.1 (0.8-1.7)	0.56	1.4 (0.8-2.4)	0.18	1.0 (0.7-1.6)	0.84	1.3 (0.7-2.3)	0.38
Kidney involvement	0.9 (0.6-1.3)	0.61	1.0 (0.6-1.6)	0.91	0.8 (0.5-1.2)	0.30	0.7 (0.4-1.2)	0.20
Liver involvement	1.3 (0.9-2.0)	0.20	1.4 (0.8-2.5)	0.20	1.1 (0.7-1.8)	0.73	1.2 (0.6-2.1)	0.62
>1 organ involved	1.9 (1.3-2.8)	**<0.01**	1.7 (1.0-2.8)	**0.05**	1.5 (1.0-2.4)	**0.06**	1.2 (0.7-2.2)	0.43
IFE positive	1.1 (0.7-1.5)	0.81	1.4 (0.9-2.3)	0.19	1.1 (0.7-1.7)	0.67	1.3 (0.7-2.3)	0.36
Abnormal FLC ratio	1.2 (0.7-2.0)	0.49	0.9 (0.5-1.5)	0.66	1.2 (0.7-2.2)	0.49	0.9 (0.5-1.9)	0.85
dFLC >180 mg/L	1.6 (1.0-2.4)	**0.05**	1.3 (0.8-2.3)	0.34	1.7 (1.0-2.9)	**0.04**	1.5 (0.8-2.8)	0.23
Abnormal HLC ratio	0.9 (0.6-1.3)	0.48	1.2 (0.7-2.0)	0.54	0.9 (0.6-1.4)	0.70	1.3 (0.7-2.4)	0.40
HLC immunoparesis	1.0 (0.6-1.7)	0.96	0.9 (0.5-1.8)	0.77	1.1 (0.6-2.1)	0.73	1.1 (0.5-2.4)	0.83
HLC immunoparesis (moderate)	1.0 (0.7-1.4)	0.92	1.1 (0.7-1.7)	0.74	1.0 (0.6-1.5)	0.85	1.1 (0.6-1.9)	0.83
HLC immunoparesis (severe)	1.4 (0.9-2.3)	0.16	1.6 (1.0-2.9)	**0.09**	1.7 (1.0-2.9)	**0.06**	2.4 (1.2-4.6)	**<0.01**
Total Ig immunoparesis	1.1 (0.7-1.6)	0.80	1.0 (0.6-1.6)	0.91	1.2 (0.7-2.0)	0.44	1.3 (0.7-2.4)	0.42
Total Ig immunoparesis (moderate)	1.2 (0.8-1.8)	0.39	1.1 (0.7-1.8)	0.77	1.3 (0.8-2.0)	0.33	1.2 (0.7-2.2)	0.48
Total Ig immunoparesis (severe)	0.9 (0.5-1.6)	0.65	0.9 (0.4-1.9)	0.78	0.9 (0.5-1.8)	0.81	1.0 (0.4-2.3)	0.93
Multivariate								
Heart involvement	1.6 (0.9-2.7)	0.12	1.7 (0.8-3.2)	0.14	-	-	-	-
NT-proBNP >332 ng/L	1.8 (0.8-4.3)	0.16	2.1 (0.8-5.1)	0.14	-	-	-	-
>1 organ involved	1.7 (1.0-2.9)	0.06	1.0 (0.5-2.1)	0.93	1.8 (1.1-2.9)	**0.02**	-	-
dFLC >180 mg/L	1.5 (0.9-2.5)	0.13	-	-	1.7 (1.0-2.8)	**0.05**	-	-
HLC immunoparesis (severe)	-	-	2.0 (1.0-4.2)	0.07	1.8 (1.0-3.1)	**0.05**	-	-

HR: hazard ratio; CI: confidence interval