Cardiac biomarkers are prognostic in systemic light chain amyloidosis with no cardiac involvement by standard criteria

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Word count: main text-3723, abstract- 249, tables- 4, figures- 3, references- 20

Acknowledgements: We would like to thank all those at the National Amyloidosis Centre who helped with the clinical care of the patients' involved in this study.

Abstract

Patients with systemic AL amyloidosis with no evidence of cardiac involvement by consensus criteria have excellent survival, but 20% will die within 5 years of diagnosis and prognostic factors remain poorly characterised. We report the outcomes of 378 prospectively followed Mayo Stage I patients (N-terminal pro b-type natriuretic peptide <332 ng/L, high sensitivity cardiac troponin <55ng/L). The median presenting N-terminal pro b-type natriuretic peptide was 161 ng/L, high sensitivity cardiac troponin 10 ng/L, creatinine 76 µmol/L and mean left ventricular septal wall thickness, 10mm. Median follow up was 42 (1-117 months), with 71 deaths; median overall survival was not reached (78% survival at 5 years). Although no patients had cardiac involvement by echocardiogram, a proportion (N=25/90, 28%) had cardiac involvement by cardiac magnetic resonance imaging. Age, autonomic nervous system involvement, N-terminal pro b-type natriuretic peptide >152 ng/L, high sensitivity cardiac troponin >10 ng/L and cardiac involvement by magnetic resonance imaging were predictive for survival; on multivariate analysis only N-terminal pro b-type natriuretic peptide >152 ng/L (p=<0.008, HR 3.180, CI=1.349-7.495) and cardiac involvement on magnetic resonance imaging (p=0.026, HR=5.360, CI=1.219-23.574) were prognostic. At 5 years, 70% of patients with N-terminal pro b-type natriuretic peptide >152 ng/L were alive. In conclusion, N-terminal pro b-type natriuretic peptide is prognostic for survival in patients with no cardiac involvement by consensus criteria and cardiac involvement is detected by magnetic resonance imaging in such cases. This suggests that N-terminal pro b-type natriuretic peptide thresholds for cardiac involvement in AL amyloidosis may need to be redefined.

Introduction

Systemic immunoglobulin light chain amyloidosis (AL) is characterised by the extracellular deposition of misfolded immunoglobulin light chains resulting in progressive organ dysfunction. Patient outcomes are largely dependent upon the severity and pattern of organ involvement.[1] Accurate stratification of patients is needed to assess prognosis and to facilitate treatment decisions. Cardiac involvement is *the* critical determinant of survival. NT-proBNP (N-terminal pro b-type natriuretic peptide) is a remarkably sensitive marker of cardiac involvement and is one of the cornerstones of the international amyloidosis consensus group diagnostic criteria for cardiac involvement.[2] Change in NT-proBNP is crucial in monitoring the effect of therapy in patients with cardiac amyloidosis.[3] These findings have followed from the seminal work by the Mayo clinic group discovering NT-proBNP and TNT (troponin T) as sensitive biomarkers for prognosis in AL[4] and the development of the 2004 Mayo prognostic scoring system, which has been further refined in 2012.[5] The widely used 2004 staging system uses thresholds of NT-proBNP <332 ng/L and a TNT <0.035 µg/l to classify patients into stage I, II or III if both biomarkers are normal, one biomarker elevated or both biomarkers elevated respectively.[4] This is with progressively poorer prognosis (median survival of 27.2, 11.1 and 4.1 months respectively). Lately, with the move to high sensitivity troponin T (hsTNT), the threshold for troponin is <55 ng/L.

Recent studies of patients with normal NT-proBNP and hsTNT without cardiac involvement, (so called Mayo stage 1 disease) show excellent outcomes with median overall survival (OS) not reached at 5 years. There are still deaths in this group of patients and few have explored factors predictive of poor survival. There are a number of novel prognostic variables in AL including: number of organs involved, a high percentage of bone marrow plasma cells,[6] raised von Willebrand factor[7] and high growth differentiation factor-15 levels.[8] None of the studies have focused specifically on the stage I patients. Liver involvement is widely believed to contribute to the poor prognosis of such cases but in the vast majority of cases this is associated with other organ involvement.[9]

We designed this study to assess prognostic variables in patients with systemic AL amyloidosis who had no evidence of cardiac involvement by echocardiographic criteria and who had normal cardiac biomarkers (Mayo 2004 stage 1).

Methods

This study included all prospectively followed up patients with AL amylodoisis from an ongoing prospective observational study (Alchemy) from 2009-2017, with Mayo Stage 1 disease (defined by normal cardiac biomarkers (NT-proBNP <332 ng/L, hsTnT <55 ng/L)). A threshold of hsTnT of 55 ng/L was used (equivalent to 0.035 μg/L cTnT) and this has been used by our laboratory since we moved from standard troponin-T measurements to using hsTNT measurements at our centre.

A diagnosis of amyloidosis was confirmed by Congo-red staining of a tissue biopsy, with the demonstration of characteristic birefringence under cross polarized light, and AL typing was confirmed by immunohistochemistry, with specific antibodies or by mass spectrometry. Hereditary amyloidosis was excluded by appropriate gene sequencing, if there was a doubt about the diagnosis of AL. As part of the study protocol, all patients had a detailed baseline assessment of organ function, including biomarker measurements and imaging with echocardiogram and ^{123}I-labelled serum amyloid P (SAP) scintigraphy. Organ involvement was defined according to the international amyloidosis consensus (ISS) criteria.[2] Specifically, the echocardiogram was considered to show cardiac involvement if the patients had mean left ventricular (LV) wall thickness >12 mm, in absence of any other cause of left ventricular hypertrophy. NT-proBNP was <335 ng/L and high sensitivity cardiac troponin T (hsTNT) <55 ng/L in all cases. Cardiac magnetic resonance imaging (CMR) was added to routine baseline assessments from late 2015 onwards and the result of the baseline CMR was recorded, where available. A typical pattern of late gadolinium enhancement and an extracellular volume (ECV) >0.30 on an MRI were used as criteria suggestive of cardiac involvement by CMR.[10]

Overall survival (OS) was calculated from date of diagnosis to death or last follow-up. Factors were analysed for their impact on survival and this included: age, sex, type and number of organ involvement, difference in serum free light chains (dFLC) and markers of cardiac, renal and liver function and

treatment given. Since asymptomatic liver involvement is often detected by ^{123}I -SAP scintigraphy [11] we assessed the prognostic significance of amyloid load by this imaging method. Survival outcomes were analysed using the Kaplan-Meier method with comparisons done using the log rank test. All p-values were two sided with a significance level of < 0.05 and median values were used to dichotomise continuous variables. Any factors found to be significant on univariate analysis were further assessed in multivariate modelling by Cox's regression analysis. Statistical analysis was performed using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp).and Stata (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). Approval for analysis and publication was obtained from the NHS institutional review board and written consent was obtained from all patients in accordance with the Declaration of Helsinki.

Results

A total of 378 patients were included in this study. The patient baseline characteristics are outlined in table 1. The median patient age was 69 years (range 35-92 years); 212 (56.1%) were men. The median number of organs involved was 2 (range 1-7). *None* of the patients had cardiac involvement by standard criteria.[12] The majority of patients had renal involvement (n=277, 73.3%). Thirty-nine patients (10.3%) had liver involvement by ISS criteria, whilst liver was abnormal by ^{123}I-SAP scintigraphy in 111 (29.4%). By ^{123}I-SAP scintigraphy, amyloid deposition was seen in 255 patients with the distribution: no amyloid in 122 patients (32.4%); 181 patients (48.0%) had a small or moderate amyloid load and 74 (19.6%) had a large amyloid load. The mean LV wall thickness was 10mm (range 6-13mm). Six patients had a mean LV thickness of 13mm, but none with echocardiogram appearances suggestive of cardiac amyloidosis based on their preserved global strain pattern. In all six patients the NT-proBNP was <335 ng/L, and co-existing hypertension was present in 5/6. The median NT-proBNP was 161 ng/L (range 8-330 ng/L) and hsTNT was 10 ng/ml (range 3-51 ng/L). Peripheral and autonomic neuropathy were seen in 43 (11.4%) and 30 (7.9%) cases respectively.

The median follow up was 42 months (1-117 months). There were 71 deaths. Median OS was not reached (Figure 1A). The OS at 1, 3, and 5 years was 96%, 87% and 78% respectively. Liver

involvement by ISS (ALP > 1.5 x upper limit of normal (ULN)) was not prognostic for survival (p=0.204, HR=1.518 CI=0.797-2.891), neither was any abnormality in the ALP (defined by an ALP outside the ULN of 129U/L) (p=0.753, HR= 0.923, CI=0.561-1.519) (Figure 1B). Although liver involvement was detected more frequently on SAP scintigraphy, neither liver involvement by SAP (p=0.284, HR=0.750, CI=0.443-1.269), nor the amyloid load on SAP scans (p=0.894, HR=0.956, CI= 0.489-1.869) were prognostic for survival. Renal involvement was not predictive of outcome using the standard consensus criteria definition,[12](p=0.396, HR=0.804, CI=0.486-1.330), or an eGFR of <30 mls/min (p=0.483, HR=2.11 CI=0.262-17.047), but only 14 patients had an eGFR <30mls/min and only 5 patients had an eGFR <20mls/min. Patients with autonomic nervous system involvement had significantly poorer outcomes on univariate analysis (p=0.018, HR=2.177, CI=1.144-4.142), but patient numbers were small. Age was predictive of survival on univariate analysis (p=0.005, HR=1.034, CI=1.010-1.059) but using receiver operating characteristic (ROC) analysis there was no clearly identifiable threshold for poorer outcomes. The presenting free light chains were not prognostic for survival in this cohort as a continuous variable or a dichotomous variable above or below a dFLC of 50mg/L or 180mg/L (table 1). At four years 83% versus 77% of patients with a dFLC above or below a value of 50mg/L were alive (log rank p= 0.202).

Although all the patients included in this study had no evidence of cardiac involvement, and cardiac biomarkers below the threshold for defining cardiac involvement, hsTNT and NT-proBNP were still prognostic for survival both on univariable analysis and only NT-proBNP on multivariate analysis. We undertook ROC analysis to define thresholds for NT-proBNP and hsTNT, (identified as 152 ng/L and 10 ng/L respectively), as prognostic cut offs for poorer survival. The OS was significantly better for patients with NT-proBNP <152 ng/L vs. those with a greater value (although median OS not reach for either group) (log rank p=<0.001; figure 1C). At 1, 3, and 5 years, for patients with NT-proBNP below and above 152 ng/L, the OS was 96% vs 94%; 91% vs 82%; and 83% vs 70% respectively. The OS at 1, 3, and 5 years for patients with hsTNT below and above 10 ng/L was 98%% vs 93%%, 91% vs. 84% and 87% vs 70% respectively. The median OS was not reached for either group. There was no significant difference in the median creatinine or eGFR for patients with a NT-proBNP value </ ≥

152ng/L (p=0.091 and 0.206 respectively) ruling out impairment of renal function as a cause of abnormal NT-proBNP in this cohort.

CMR was undertaken since 2015 and results were available on 90/378 (24%) patients. Twenty-eight percent (n=25/90) of patients had cardiac involvement by CMR. In the patients who had a CMR with NT-proBNP below (32 patients) and above (58 patients) 152 ng/L, the CMR was positive for amyloid deposition in 22% vs 31% of cases, respectively (p=0.353) (see Table 2). There was a trend towards higher NT-proBNP in patients with a positive CMR median NT-proBNP 220 ng/L vs. 169 ng/L (p=0.089) (Figure 2). The median LV wall thickness by echocardiogram (11mm vs. 10mm (p=0.1902)) and hsTNT values (17 ng/L vs. 14 ng/L (p=0.373)) were not significantly different in those patients with CMR positivity for amyloid deposition compared to those patients with negative CMR findings respectively. After gadolinium contrast, the extracellular volume fraction (which directly reflects myocardial interstitial expansion by amyloid deposition) was calculated with a median ECV of 0.33 (0.24-0.71). The mean ECV of patients with cardiac involvement was 0.44 vs. 0.31 (p<0.0001) for those without cardiac involvement. Cardiac involvement on CMR was prognostic for OS with the 1 and 2 year survival for patients with CMR positive vs. negative being 86% vs 98% and 69% vs 98% respectively (p= 0.007, HR=6.563, CI=1.689-25.492) (Figure 1D). Too few patients have sufficient follow up for meaningful longer-term survival analysis at present.

Treatment details were available in 97% of cases (n=368/378) and are outlined in table 1. A total of 91% (n=346/378) patients were treated with chemotherapy. The most common treatment given was bortezomib (mostly cyclophosphamide-bortezomib-dexamethasone) (N= 246/368, 67%) followed by thalidomide (mainly cyclophosphamide-thalidomide-dexamethasone) (N=110/369, 30%). Fifteen percent (N=55/368) of patients has an upfront autologous stem cell transplant (ASCT). Treatment type was not prognostic for survival on univariate analysis (table 1).

In the 346 patients who received chemotherapy 89% (N=337/378) were evaluable at six months. Haematological response was as follows: complete response (CR) 51% (N=173/378, very good partial response (VGPR) 13% (N=46/346), partial response (PR) 3% (N=12/346), no response (NR) 4% (N=14/346) and progressive disease (PD) 17% (N=58/346). The overall survival of patients who

achieved a CR to treatment was significantly longer than those who did not achieve a CR (median OS 109 vs 75 months, p <0.001). The six-month landmark analysis was as follows: CR- median survival not reached, non-CR median survival 88 months, p <0.001. Survival at one and three years by NT-proBNP <152ng/L was: CR=100%, 96% vs non-CR: 90%, 69% respectively, and for patients with NT-proBNP >152ng/l: CR= 96%, 80% and non-CR: 91%, 53% respectively, p= <0.001. A total of 95 patients had NT-proBNP >152ng/L and achieved a CR at six months. Of these patients, 15% (N=14/95) achieved a reduction in the iFLC to <10mg/L at six months. There was no significant survival difference between those patients who achieved an iFLC <10mg /L at 6 months versus those who did not (p=0.396). Of the 95 patients with NT-proBNP >152ng/L who achieved a CR at six months, 8% (N=8/95) achieved a CR at one month, and 39% (N=37/95) after three months. There was no significant difference in overall survival between those patients who achieved a CR versus non-CR at one month (p=0.281), or three months (p=0.402).

Of the 346 patients treated, 80% (N=277/346) had NT-proBNP readings at 12 months. Based on a 30% change in NT-proBNP to define response: 32% (N=88/277) patients had reduction in their NT-proBNP levels, 50% (N=138/277) patients' values increased and 18% (N=51/277) patients did not reach either criteria. When analysing the entire cohort there was no significant difference in survival between patients who had an NT-proBNP response versus no response/ progression, (p=0.193); the 3 year survival of patients was 76% versus 70% for patients with an NT-proBNP response compared with unchanged/progression, respectively. However, when the analysis was restricted to patients with NT-proBNP >152 ng/L, outcomes were significantly poorer in the patients with a baseline NT-proBNP level of >152ng/l who progressed (p= 0.001).

Multivariate models were developed using variables significant on univariate analysis, defined as a p value <0.05, (table 3). A model including CMR was done separately due to the limited number of patients with CMR data. On multivariate model including age, autonomic nervous system involvement, NT-proBNP >152 ng/L, hsTNT >10ng/L, only NT-proBNP (p=0.008, HR=3.180, CI=1.349-7.495) was an independent predictor of survival, (table 1). When cardiac involvement by MRI was added to the

model, only cardiac amyloid on CMR (p=0.026, HR=5.360, CI=1.219-23.574) remained an independent predictor of outcome.

The cause of death was available for 20/71 patients (28.2 %). The most common cause of death was progressive amyloidosis (5 patients), end stage renal failure (4 patients), and pneumonia (3 patients). Two patients died of splenic haemorrhage and two due to complications of treatment. One patient each died of a fall, heart failure, sepsis and a fatal arrthymia respectively. Of the 71 patients who died, 82% (n=58/71) had a repeat echocardiogram. In 12% (n=7/58) cases the echocardiogram was clearly suggestive of cardiac amyloid progression based on an lVS >12mm and a reduced global strain pattern. In 57% (n=4/7) of these patients their baseline NT-proBNP was above our threshold of 152ng/l suggesting that in at least a proportion of patients the cause of death was progressive cardiac amyloidosis.

Discussion

Patients with AL amyloidosis without cardiac involvement by the consensus criteria have excellent outcomes. These patients have normal cardiac biomarkers and therefore, by definition, have Mayo (2004) stage one disease. Whilst this study confirms the excellent long-term outcomes of patients with this early disease, 22% of patients died within five years of diagnosis. We report here that cardiac biomarkers remain prognostic even in this group of patients at a lower threshold (NT-proBNP < 152 ng/L) than previously outlined. We also show that patients with AL amyloidosis have CMR scans showing cardiac involvement, with adverse prognostic implications, even in patients with low biomarker levels and with echocardiogram features not suggestive of amyloidosis.

Cardiac involvement in AL amyloidosis is currently defined by both echocardiogram criteria (>12mm mean wall thickness in diastole by echocardiogram in absence of other causes of LVH) and by elevation of the cardiac biomarker (NT-proBNP >332 ng/l), in the absence of renal failure or atrial fibrillation. NT-proBNP is unquestionably one of most sensitive markers of cardiac stress in AL reflecting the direct pathological activity of amyloidogenic light chains/toxic oligomers, mediated by activation of the p38-MAP kinase pathway. The importance of NT-proBNP for defining cardiac involvement is reflected in

the initial Mayo staging scoring system where a threshold for NT-pro-BNP was defined using a multivariate model with a value of 332 ng/l (the upper reference limit of normal for women older than 50 years) providing the best fit and the highest hazard ratio (table 4).[4] The prognostic importance of this value has since been confirmed in a number of studies although the threshold value itself has never been systematically re-examined. In 2011 we reported a small cohort of patients with NT-proBNP <127 ng/L had much better outcomes and those with NT-proBNP >127 ng/L had a higher risk of developing cardiac amyloidosis on longer term follow up.[13] In the 2011 cohort, we had not access to MRI scanning understand the relevance of these findings. Dittick et.al (2019) have also highlighted the difficulty of using current Mayo staging scores in the setting of renal impairment and atrial fibrillation.[14] The Mayo Clinic data, and data from the international collaborative series, were also generated in the era where highly effective novel agent-based therapies were not routinely available. The survival of patients with stage one disease in these earlier series may now be considered relatively poor compared with contemporary survival outcomes – allowing for a potential opportunity to revisit the NT-proBNP threshold for defining cardiac involvement.

This current data suggests that the extreme sensitivity of NT-proBNP in AL amyloidosis extends to a much a lower value of 152 ng/L and patients with a subtle increase in NT-proBNP (>152ng/L) had poorer outcomes (HR=3.180 (CI 1.329-7.495)). The "normal" range for NT-proBNP is between 100-125 ng/L for those aged less than 70 years which is lower than the prognostic threshold identified in this cohort. Other factors can influence NT-proBNP levels such as age. There was a correlation of NT-proBNP with age (P= 0.002) but there was no significant difference in the numbers of patients over or below 75 years with NT-proBNP < or > 152 ng/L. Additionally, age was not significant in the multivariable analysis.

The exquisite prognostic sensitivity of NT-proBNP in AL amyloidosis may suggest either early cardiac involvement or light chain proteotoxicity. The structurally established echocardiographic criteria for AL cardiac involvement is an LV wall of >12 mm, (in absence of other causes). It is conceivably possible for a patient with baseline 8-10mm LV wall could have substantial amyloid deposition before the threshold of 12mm is reached. The opportunity to track changes in NT-proBNP during development

of cardiac AL is rare. The kinetics of NT-proBNP increase as well as its correlation with LV wall thickness at early stage of the disease process remain largely unknown.

CMR is an alternative method of monitoring patients with cardiac amyloidosis. In this current cohort, a third of all patients who had a CMR showed features of cardiac amyloidosis. Moreover, the presence of amyloid deposition on CMR was an independent prognostic marker. CMR, with late gadolinium enhancement (LGE) and T1 mapping, is emerging as a highly sensitive and specific tool for diagnosis and characterisation of cardiac amyloidosis in AL (Figure 3). [15] Transmural LGE with phase-sensitive inversion recovery (PSIR) is associated with the burden of cardiac amyloid and predicts death independent of NT-pro-BNP and other known prognostic factors.[10] In this cohort, it clearly identified cardiac involvement in patients where the echocardiogram was not suggestive of cardiac amyloidosis but not all patients with NT-proBNP >152 ng/L had abnormal CMR (31% had abnormal CMRs) and not all patients with NT-proBNP <152 ng/L had normal CMRs (22% had abnormal CMRs). This suggests that CMR provided complementary information on patients' cardiac damage. NT-proBNP may be detecting cardiac damage by light chain proteotoxicity before structural amyloid deposition is apparent on CMR, conversely, some patients may have non-proteotoxic light chains (analogous to cardiac amyloid deposition in ATTR (transthyretin) amyloidosis) where the structural changes are apparent on CMR before biomarkers become abnormal. In this early stage of the disease, NT-proBNP and CMR findings should be used together for defining cardiac involvement.

In this study, liver involvement, a previously reported poor prognostic marker,[9,16] did not significantly impact survival. Relatively few patients had significant liver involvement – only 10% by consensus criteria (although a third had asymptomatic liver involvement on ^{123}I-SAP scintigraphy). The strict exclusion of cardiac involvement by consensus criteria may have excluded patients with advanced liver involvement since the latter patients often have multi-organ amyloidosis. Likewise, although the majority of patients had renal involvement, 277 (73.3%) the median presenting creatinine was low (76 µmol/L), with only a small proportion (N=14/375, 3.7%) with an eGFR < 30 mls/min, which may explain why neither the presence of renal involvement nor proteinuria was a predictor of survival.

Autonomic involvement (ANS) was significant on univariate but not multivariate analysis, but the number of patients was small.

This study has limitations and needs to be interpreted in this context. This is single centre data but we are planning validation in an international collaborative data set. One major limitation is that the exact cause of death was only available in a small proportion of patients and when the cause was recorded as "amyloidosis" this does not elucidate whether cardiac amyloidosis was the real cause of death. Progressive cardiac amyloidosis does appear to be the cause of death in at least a proportion of patients in this study, based on serial echocardiogram imaging. The use of a very sensitive marker of cardiac disease like NT-proBNP at a low level is also challenging as other unrelated factors can impact upon NT-proBNP (such as age, renal function, sex, body mass index as evidenced by the Framingham study from 2011, and a more recent study by Dittrick et al. (2019).[14,17] Finally, only a proportion of patients had CMR scans. Larger studies are needed to address these limitations.

In conclusion, this study demonstrates that in patients with AL amyloidosis with no cardiac involvement by consensus criteria even small elevations of NT-proBNP as well as cardiac involvement by CMR are factors highly prognostic for survival. This novel finding offers some insight into the heterogeneity in survival of Mayo Stage 1 patients. There findings have implications for clinical practice. We suggest that a baseline cardiac MRI scan should be considered at diagnosis for stage I AL patients, if possible. Better outcomes for patients in a CR and those with decrease in NT-proBNP, suggest that in "high risk" stage 1 patients (those with NT-proBNP >152 ng/L) the goal of therapy should be similar to those with cardiac AL i.e. a complete haematological response. The follow up of such patients should include routine NT-proBNP measurement including assessment of response (as patients with presenting NT-proBNP >152ng/L and NT-proBNP progression (>30% increase) had poorer outcomes); those with NT-proBNP progression should be considered for further treatment. The "high risk relapse criteria" defined by the Italian amyloidosis group, should be applied for treatment at relapse for patients with NT-proBNP >152 ng/L (high risk stage I). [18] Serial CMR data is needed to assess cardiac structure and functional changes to delineate the natural history of 'high risk' patients and to help identify interventions to prevent progressive cardiac involvement.

Conflict of Interest: none of the authors have any conflicts of interests to declare.

Author contributions: FAS and ADW conceived study, analysed data and wrote manuscript. MF, AMN, RM, SM. SS, HJL, JDG, CJW and PNH all contributed to the manuscript and provided critical input. All authors reviewed the final version of the manuscript.

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Table 1: Baseline patient characteristics, (total patients, N=378) including univariate analysis.

Factor assessed for significance	Median (range), N(%)	HR (CI)	Cox regression P value
Age (years), >70 years	69 (35-92), 93 (25)	1.034(1.010-1.059)	**0.005**
Male sex	212 (56.1)	0.850(0.667-1.082)	0.186
Number of organs involved	2 (1-7)		
Renal	277 (73.3)	0.804 (0.486-1.330)	0.396
PNS	43 (11.4)	1.612 (0.866-3.000)	0.132
ANS	30 (7.9)	2.177 (1.144-4.142)	**0.018**
Soft Tissue	44 (11.7)	1.792 (0.982-3.273)	0.057
GI	36 (9.5)	1.428 (0.731-2.789)	0.297
Spleen	160 (42.3)	1.279 (0.759-2.154)	0.354
Renal parameters			
Creatinine (µmol/L)	76 (27-487)	1.004 (1.000-1.008)	0.036
eGFR (ml/min)	69 (18- >90)	0.990 (0.972-1.008)	0.274
eGFR < 30ml/min	14 (3.73)	2.11 (0.262-17.047)	0.483
Proteinuria (g/24h)	4.28 (0.03- 58.46)	0.99 (0.997-1.001)	0.198
Liver parameters			
Albumin (g/L)	32 (15-50)	0.994(0.968-1.020)	0.633
Bilirubin (mmol/L)	5 (1-57)	1.00(0.998-1.001)	0.630
ALP (U/L	77 (31-2112)	0.923 (0.561-1.519)	0.753
Abnormal ALP (<129U/L)	47 (22.9)	0.872(0.352-2.155)	0.766
Liver involvement (ALP 1.5x upper limit)	39 (10.3)	1.518 (0.797-2.891)	0.204
SAP liver involvement	111 (29.4)	0.750 (0.443-1.269)	0.284
SAP load None/equivocal Small/moderate Large	 122 (32.4) 181 (48.0) 74 (19.6)	 0.956(0.489-1.869)	0.894
Cardiac parameters			
NT-pro-BNP (ng/L)	161 (8-330)	1.006 (1.003-1.009)	**<0.001**
NT-pro-BNP >152 (ng/L)	208 (55)	2.413 (1.448-4.021)	**0.001**
hsTNT (ng/L)	10 (3-51)	1.032 (1.011-1.054)	**0.003**
hsTNT >10 (ng/L)	76 (37.1)	1.249(0.554-2.813)	**0.592**
Echocardiogram (mean LVW)	10 (6-13)	0.998(0.820-1.215)	0.984
Haematological parameters			
Presenting kappa (mg/L)	22.55 (1.5 -935)	1.101 (0.847-1.203)	0.916
Presenting lambda (mg/L)	26.6(1.9- 6180)	0.991 (0.831-1.181)	0.917
dFLC (mg/L)	1.40 (0.1- 6064)	0.991 (0.831-1.181)	0.919
dFLC > 50mg/L	104 (28.2)	1.431 (0.859-2.384)	0.202
dFLC >180mg/l	51 (13.5)	1.590(0.848-2.979)	0.143
Treatments			
PI based	248 (67.4)	0.732 (0.417-1.287)	0.279
Imid based	164 (44.6)	1.560 (0.937-2.599)	0.088
Alkylator	43 (11.7)	1.084 (0.529-2.224)	0.825
ASCT	55 (14.9)	0.476 (0.143-1.591)	0.137
No treatment/ trial treatment*	24 (6.5)		
Missing data	10 (2.6)		
Treatment interval			
2008-2012	29 (8.4)		
2012-2016	88 (25.5)		
2014-2016	80 (23.2)		
2016-2018	77 (22.3)		
No treatment/ missing data	33 (9.6)		

*Trial treatment MLN9708. PNS, peripheral nervous system ; ANS, autonomic nervous system ; GI, gastrointestinal; NT-pro BNP, N-terminal pro b-type natriuretic peptide; hsTNT, high-sensitive cardiac troponin T; dFLC, difference between involved and uninvolved serum free light chains; ALP, alkaline phosphatase; SAP, ^{123}I labelled serum amyloid P component (SAP) scintigraphy; LVW, left ventricle wall; eGFR, estimated glomerular filtration rate, Imid=immunomodulatory therapy, PI= proteasome inhibitor.

Table 2: A comparison of patients with N-terminal pro b-type natriuretic peptide >152ng/L vs <152ng/L.

	NT- proBNP ≤152 g/L (N=170)	**NT-pro BNP >152 ng/L (N=208)**	**P value***
Other biomarkers:			
High-sensitive cardiac troponin T	7	11	**<0.001**
dFLC	10.90	18.70	0.204
ALP (U/L)	170	207	0.994
Cardiac magnetic resonance imaging (CMR) findings:			
CMR positive for amyloidosis (N=90)	7(22%)	18(31%)	0.364
Extracellular volume	0.327	0.355	0.470
Echocardiogram parameters:			
Echo global strain (%)	-21.96	-20.34	0.40
Echo lVS (mm)	10	10	0.914

*Mann-Whitney U test for non-parametric variables; Chi-squared for categorical variables. NT-pro BNP, N-terminal pro b-type natriuretic peptide; ALP, alkaline phosphatase; CMR, cardiac magnetic resonance imaging; lVS, interventricular septal thickness; dFLC, difference between involved and uninvolved serum free light chains

Table 3: Factors included in a multivariate analysis and their significance (separate multivariate models were developed with and without CMR due to smaller patient numbers with CMR data).

	Analysis *excluding* CMR findings	**Analysis *including* CMR findings**
Factor in multivariate analysis	**P value/ HR (CI)**	
Age	0.269/1.021(0.984-1.058)	0.363/0.967(0.900-1.039)
ANS	0.624/0.696(0.164-2.962)	0.322/6.749(0.154-295.885)
NT-proBNP> 152ng/L	**0.008**/3.180(1.349-7.495)	0.918/1.074(0.999-1.154)
hsTNT >10ng/L	0.771/0.880(0.370-2.091)	0.073/1.059(0.995-1.128)
CMR positivity	/	**0.026/5.360(1.219-23.574)**

HR, hazard ratio; CI, confidence interval; ANS, autonomic nervous system; NT-proBNP, N-terminal pro b-type natriuretic peptide; hsTNT, high-sensitive cardiac troponin T; IMiD, immunomodulatory drug; CMR, cardiac magnetic resonance imaging

Table 4 : A review of the literature to outline previous studies and the previous prognostic thresholds of NT-proBNP

Study details	*NT-proBNP threshold*	*Survival*
Palladini G *et. al.* 2003 [19]	152 pmol/L= 1288ng/L	7.6 per 100 person-years (95% CI, 3.6 to 15.7) and 72.2 per 100 person-years (95% CI, 54.2 to 86.1)
Dispenzieri A. et.al 2004 [4]	332 ng/L	<332pg/ml survival 20 months >332pg/ml 5.8 months
Kumar SK et.al 2011[20]	332 ng/L	Median OS from diagnosis for patients NT proBNP <332ng/L was 4.0 years vs 2.4 years if either NT-proBNP was >332ng/L or cTnT >0.035 µg/L.
Wechalekar AD et. al 2011 [13]	NT-proBNP <15 pMol/L= 127 ng/L	5-year survival 98% *versus* 88% for those above and below respectively
Kumar S et.al. 2012[5]	1,800 pg/mL= 1800ng/L	NT-ProBNP ≥ 1,800 pg/mL was 10.5 months, compared with median not reached for those with NT-ProBNP < 1,800 pg/mL

Figure Legends

Figure 1: Survival curves for Mayo stage 1 patients demonstrating: **1A:** Overall survival was not reached; overall survival at 1, 3, and 5 years was 95%, 87% and 76% respectively; **1B** : The impact of haematological response to treatment at six months and survival outcomes, patients achieving a complete response to treatment versus not a complete response (log rank P <0.001)); **1C :** N-terminal pro b-type natriuretic peptide (NT-pro-BNP) above and below 152 ng/L showing poorer outcome for patients with NT-proBNP >152 ng/L, (log rank p= 0.001); **1D :** Cardiac magnetic resonance imaging findings demonstrating a significantly poorer outcome for patients with cardiac amyloid deposition, (log p= 0.007)

Figure 2: The difference in N-terminal pro b-type natriuretic peptide (NT-pro-BNP) between patients with, and without, evidence of cardiac involvement on cardiac magnetic resonance imaging (CMR).

Figure 3: CMR image of a patient with no evidence of cardiac amyloidosis by echocardiogram and NT-BNP <332 ng/L showing characteristic features of cardiac involvement: 3A: Four-chamber steady state free precession (SSFP) cine (top right panel); 3B: corresponding native T1 map (top left panel) with an elevated value of 1209m; 3C: corresponding phase sensitive inversion recovery late gadolinium enhancement (PSIR LGE) image showing subendocardial LGE (bottom right panel); 3D: corresponding extracellular volume (ECV) map with an elevated value of 0.47 (bottom left panel)