Clinical characteristics and prognosis of cardiac amyloidosis defined by mass spectrometry-based proteomics in an Australian cohort.

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Introduction

Amyloidosis is a disease of constitutive protein misfolding resulting in extracellular deposition of pathological, insoluble, β-sheet fibrillar bundles that destroy the structure and function of affected organs and tissues. Cardiac infiltration traditionally implies a very poor prognosis[1-4]. The major systemic amyloidoses associated with cardiac deposition[5, 6] are primary light chain amyloidosis (AL amyloidosis) arising from abnormal immunoglobulin light chain deposition; wild-type amyloidosis (ATTRwt) resulting from hepatic-derived wild type transthyretin accumulation; and hereditary or familial amyloidosis (ATTRv) due to deposition of mutant transthyretin proteins. The main constituent fibril protein in tissue deposits determines the amyloid subtype. The importance of correct subtype classification cannot be overstated, due to significant differences in the treatment and prognosis of the various amyloid subtypes. Immunohistochemistry (IHC) on tissue samples has been recommended to assist in diagnosis, however it is largely unreliable outside of highly specialised centres such as the United Kingdom National Amyloid Centre (NAC)[7-11].

Laser microdissection and tandem mass spectrometry (LMD-MS)-based proteomic analysis has been demonstrated to have 98% accuracy for subtype classification

compared with 42% for IHC performed on the same samples[12]. In an Australian series utilising LMD-MS on 138 biopsies from 35 organ sites (including 24 cardiac biopsies) the amyloid subtype was identified in 121 out of 131 cases (92%)[11]. Few studies have reported patient outcomes on the basis of direct fibril assessment from cardiac samples and there is limited data on the Australian experience with cardiac amyloidosis.

Given the poor reliability of IHC determination of amyloid subtype, we aimed to definitively characterise the amyloid subtype by mass spectrometry in an Australian cohort of patients with cardiac amyloidosis.

Methods

Study Design

St Vincents Hospital, Sydney is the state-wide referral centre for cardiac transplantation in New South Wales and South Australia and provides a diagnostic endomyocardial biopsy service. Patients with histologically confirmed amyloid deposition in cardiac tissue samples were identified from the Anatomical Pathology database of St Vincent's Hospital (SVH), Sydney between 1992 and 2011. This included endomyocardial biopsies collected at SVH and samples referred for review by Royal Adelaide Hospital (RAH). Amyloid deposition was confirmed by positive Congo red staining and polarisation microscopy. IHC was not routinely performed due to poor reliability and poor positive predictive value. Genetic mutation analysis was performed on selected patients.

Clinical data was collected retrospectively from the medical records at SVH and RAH, and from community medical providers. For the purposes of this study, amyloid diagnosis was considered to be at the time of endomyocardial biopsy. Demographic data, symptoms, co-morbidities and organ involvement at the time of diagnosis were noted, as well as available treatment details.

Amyloid subtype analysis by LMD-MS was performed at the Clinical Proteomics Laboratory, Mayo Clinic, Rochester as previously described[12], on diagnostic, formalin-fixed, paraffin-embedded endomyocardial tissue blocks. Briefly, paraffin sections were stained with Congo red, and amyloid deposits identified under fluorescent light were

microdissected using LMD. Microdissected fragments were digested into tryptic peptides and analysed by liquid chromatography electrospray tandem mass spectrometry (MS). MS raw data was queried using different algorithms to assign peptide and protein probability scores.

Survival was measured from the date of endomyocardial biopsy. Follow-up was closed in November 2013 with attempts made to contact all patients not reviewed within the prior 6 months, and patient survival censored at the last known date of contact.

The study was approved by the SVH Human Research and Ethics Committee (Approval Number H10/205), and the RAH Research Ethics Committee (Approval Number 111205). Informed consent was obtained from all patients alive at the time of study enrolment. An ethics application was made to the Australian Institute of Health and Welfare to obtain survival data from the National Death Index for patients biopsied prior to 2010.

Baseline Assessment

Assessment of organ involvement, symptoms, cardiac staging and plasma cell clone.

Organ involvement was noted for any patient with amyloid deposition on organ biopsy or on the basis of the following definitions. Renal involvement was defined as 24-hour urine protein excretion ≥0.5g/day or elevated creatinine (≥90umol/L in females, and ≥120umol/L in males). Hepatic involvement was defined as a serum alkaline phosphatase value >1.5 times the upper limit of the institutional reference range

(>150U/L)[13]. Peripheral nerve involvement was defined as otherwise unexplained symptoms of peripheral neuropathy. Upper gastrointestinal (GI) symptoms included dysphagia, reflux or peptic ulcer disease, nausea, vomiting, epigastric discomfort, or symptoms prompting endoscopic investigation (gastroscopy). Lower GI symptoms included constipation, diarrhoea, abdominal bloating, or symptoms prompting endoscopic investigation (colonoscopy).

Assessment of cardiac disease utilised troponin assays and from March 2009, NT-proBNP. The institutional reference ranges for NT-proBNP, troponin I and troponin T changed throughout the study period and values are reported as elevated above reference range. Troponin T results have been pooled irrespective of the use of standard or high sensitivity assay. Electrocardiogram (ECG) low voltage criteria[14] were defined as presence of QRS voltage amplitude ≤0.5mV in limb leads. Cardiac arrhythmia included self-reported or documented history of any arrhythmia or pacemaker insertion prior to diagnosis.

Evaluation for Plasma Cell Clone

Identification of monoclonal proteins in serum and urine were based on electrophoresis (EPG) +/- immunofixation electrophoresis (IFE). Free light chain (FLC) measurements were available for patients diagnosed after 2004. The institutional reference range FLC ratio is 0.26-1.65mg/L, kappa light chain 3.3-19.4mg/L, and lambda light chain 5.7-26.3mg/L. Bone marrow biopsy was reported where available, with 10% plasma cells infiltration used as the threshold of significant plasma cell burden (in keeping with

diagnostic guidelines for myeloma as per the International Myeloma Working Group (IMWG))[15].

Statistics

Statistical analyses were conducted with IBM SPSS Statistics version 22. Summary statistics are expressed as medians or frequencies (percentages). Categorical variables were compared with the Pearson's chi-squared or Fisher exact test. Independent samples t-test was used to compare normally distributed continuous variables, and a Mann-Whitney U test was utilised for non-parametric comparisons, between ATTRwt and AL groups. Values of $P \leq 0.05$ were considered significant. Overall survival was estimated using Kaplan-Meier survival curves and compared by log-rank test. Selected variables potentially impacting survival of AL patients were tested in univariate and multivariate models. A series of univariate cox regressions were performed to assess the impact on survival for each variable and any variables reaching significance were analysed together in a multivariate cox regression.

Results

Study Participants

Fifty-eight patients with amyloid deposition present on cardiac biopsy were identified (see Study Flowchart, Figure 1). Eleven patients were excluded for the following reasons: biopsy was not performed at diagnosis (e.g. autopsy biopsy) (5), patients referred from overseas and uncontactable (2), unable to be contacted (1), declined participation (1) and inadequate tissue and clinical information (1) Of the 47 patients

who were eligible for inclusion, 39 patients undergoing subtyping by laser microdissection and tandem mass spectrometry (LMD-MS) while 8 were subtyped on the basis of clinical findings and other investigations. Survival analysis was performed on the 32 AL amyloid patients and 12 ATTwt patients.

Laser Microdissection and Tandem Mass Spectrometry (LMD-MS) Subtype Analysis

Of the 47 patients, only 41 had endomyocardial biopsy tissue blocks available for LMD-MS, and two blocks were insufficient for analysis. Hence 39 of 47 (83%) patients were subtyped by LMD-MS: 11 as ATTRwt; 27 as AL (19 lambda and 8 kappa); and 1 as heavy chain (AH) amyloid. Three patients with complex presentations were clarified by the LMD-MS results (1 IgG1 heavy chain amyloid, 2 ATTRwt). The two ATTRv patients did not have sufficient tissue available for LMD-MS, and none of the patients investigated by LMD-MS were reported to have a dominant amyloid protein suggestive of hereditary amyloidosis.

Demographics, and Clinical Characteristics

The demographic data and clinical characteristics of the 47 patients (SVH 34; RAH 13) at diagnosis were compared according to subtype (Table 1). In addition to the 39 patients with LMD-MS confirmed subtype, the remaining 8 patients eligible for inclusion (ATTRv 2; ATTRwt 1; AL 5) were subtyped on the basis of clinical findings and other investigations and included in the analysis (see Figure 1).

Primary AL was diagnosed in 68% (n=32), ATTRwt in 26% (n=12), ATTRv in 4% (n=2), and AH in 2% (n=1) of the cohort. In comparison to AL/AH amyloid patients, ATTRwt patients were older (ATTRwt, 76y (69-88y); AL/AH, 60y (41-83y); P<0.01), and the majority of ATTRwt patients were male (ATTRwt, males, n=11, 92%; AL, males, n=15, 45%; P<0.001).

Median symptom duration prior to diagnosis ranged from 10-34 months. The presenting symptoms did not differ significantly between the ATTRwt and AL/AH amyloid subtypes. Multi-organ involvement, particularly renal involvement, was frequent in AL patients, occurring in 61% of patients. Other organ involvement was confirmed on tissue biopsy in AL patients: liver (1), pleural (1), pulmonary (1), muscle (1), and gastrointestinal (3). As expected, ATTRwt rarely involved other organs. A history of carpal tunnel syndrome or surgery was common across all subtypes.

Severe congestive cardiac failure symptoms (NYHA III or IV) were present at time of amyloid diagnosis in 39% of AL/AH patients and 27% of ATTRwt patients (see Table 1). A significantly higher number of ATTRwt patients had a history of arrhythmia or pacemaker insertion, compared to AL patients (ATTRwt, n=9, 75%; AL, n=9, 31%; P=0.016) and mean interventricular septal (IVS) width was also significantly higher in ATTRwt amyloid patients (ATTRwt 22mm; AL 15mm; P=0.005). Other cardiac parameters were not significantly different. All patients with ATTRwt (n=5) and AL (n=12) with available NT-proBNP results, were above the institutional reference range.

Genetic Studies

Two male patients, aged 49 and 55, were diagnosed with hereditary ATTR on the basis of clinical characteristics and heterozygosity for the transthyretin Thr60Ala (p.TTRT60A) mutation proven on sequencing. Both presented with symptomatic cardiac involvement, one had peripheral neuropathy and GI involvement at diagnosis, and the other patient went on to develop these manifestations. Neither patient reported a known family history of amyloidosis.

Evaluation for plasma cell clone

None of the ATTRwt or ATTRv patients with available records had documentation of a monoclonal gammopathy in urine or serum or an abnormal FLC ratio. Of the 32 patients with AL, 25% had a kappa restriction with median involved serum kappa light chains of 902mg/L (range 238-4470), and 75% demonstrated a lambda restriction with median involved serum lambda light chains of 299mg/L (range 79-2524). Just over one third of AL patients with bone marrow studies available had greater than 10% plasma cells on aspirate. No patient had a recorded pre-existing diagnosis of symptomatic multiple myeloma as per IMWG[15] at the time of amyloid diagnosis.

Patient Outcomes

For the 23 AL/AH patients with initial treatment recorded, the majority (n=15, 65%) received intravenous or oral melphalan plus corticosteroids, 4 (17%) received thalidomide or bortezomib containing regimens, 2 (9%) received vincristine, adriamycin

and dexamethasone combination chemotherapy, and 2 (9%) patients received colchicine. As a referral rather treating centre, adequate information was not available to assess haematological or organ response in the majority of patients studied. During the time period evaluated in this study, cardiac AL/AH amyloidosis was considered a contraindication to cardiac transplantation and in most circumstances, autologous stem cell transplantation was also contraindicated.

After a median follow-up of 63 (0.2-90) months, 7 patients remained alive (4 [33%] with ATTRwt and 3 [9%] with AL. Patients with AL had significantly inferior overall survival (OS) than the ATTRwt amyloid group (median survival 3.5 months vs. 37 months, p=0.007) (Figure 2). OS for AL was 37% at 1 year and 15% at 5 years; compared to 100% at 1 year and 44% at 5 years for ATTRwt amyloid. The patient with AH amyloid (not included in the AL survival curve) survived for 41 months. Of the two hereditary amyloid patients, one died after 44 months while awaiting combined cardiac-liver transplant and the other died after 12 months from severe autonomic, cardiac and gastrointestinal involvement precluding transplant.

The small dataset for the ATTRwt and ATTRv cohorts precluded further assessment of the impact of baseline characteristics on survival outcome. However, a variety of characteristics were analysed for the AL cohort (Table 2). Univariate cox regressions suggested that renal impairment (HR 2.48, P=0.05), NYHA class III-IV symptoms (HR 5.02, P=0.002), year of diagnosis prior to 2000 (HR 2.76, P=0.02), plasma cells ≥10% on bone marrow biopsy (HR=3.88, P=0.02), and raised troponin I >0.1ug/L or troponin

T >77ng/L (HR=4.72, P=0.02) were all associated with a significantly poorer survival. On multivariate cox regression NYHA class III-IV (HR=5.21, CI=1.21-22.49, P=0.03) and bone marrow plasma cells ≥10% (HR=3.26, CI=0.97-10.91, P=0.05) at diagnosis were the only independent variables significantly associated with worse survival.

Discussion

This study describes the characteristics and very poor survival of a cohort of patients with biopsy proven cardiac amyloidosis. To our knowledge, this is the largest Australian study characterising patients with cardiac amyloidosis. This study was performed in the era before bone scintigraphy was commonly used as part of the diagnostic approach for assessing suspected cardiac amyloid. The majority of patients (83%) were categorised on the basis of definitive amyloid subtype based on LMD-MS performed on cardiac tissue.

As expected, AL was the dominant cardiac subytpe identified in 68% of the cohort. Prognostic systems used for cardiac AL amyloidosis incorporate the cardiac biomarkers Troponin T or I and ProBNP[16, 17]. Assessment of available cardiac biomarkers in our cohort demonstrated a significant proportion of patients with poor prognosis disease. Based on the 2004 Mayo Staging System[18], 56% of AL patients tested (n=18) would be at least stage II, and of those, 7 of the 9 (78%) with proBNP and troponin assessment available were Stage III. This correlates with a median survival of 10.5-11.1 months and 3.5 months respectively which is in keeping with the median overall survival of 3.5 months in our cohort[18]. The updated Mayo Staging System by Kumar et al[16] also incorporates evaluation of the plasma cell clone utilising the Freelite FLC assay and demonstrated that a difference in FLCs (dFLC = involved FLC – uninvolved FLC), above thresholds of λ>182mg/L or κ>294mg/L, has been correlated with significantly reduced survival[19]. Our cohort demonstrated considerably higher median dFLCs of λ=297mg/L and κ= 895mg/L, highlighting that the poor prognosis of our cohort was not unexpected. In our cohort, only NHYA class III-IV and plasma cells ≥10% were

demonstrated to be significant independent predictors of inferior survival, emphasising that prognostic outcome is ultimately a function of both cardiac organ involvement and plasma cell activity/burden.

A significant proportion of the AL cohort were diagnosed prior to availability of highly effective immunomodulatory chemotherapies and were treated palliatively. In addition to the advent of immunomodulatory therapy, there is an emerging role for cardiac transplantation in advanced AL amyloidosis. An approach of induction chemotherapy, cardiac transplantation and subsequent autologous stem cell transplantation in patients with advanced AL amyloidosis and limited extracardiac disease has been demonstrated to be feasible and associated with impressive survival rates given the historically poor prognosis of these patients[20-24] and is currently under investigation in an Australian Phase II study[24].

The ATTRwt subtype was found to be relatively common comprising 26% of cardiac amyloid diagnoses, while underlying genetic mutations contributing to cardiac amyloidosis were rare (4%). The two patients with hereditary amyloidosis were diagnosed with familial amyloid polyneuropathy (FAP) and the clinical picture was typical of the cardiac predominant phenotype of the TTR T60A mutation[2, 25]. The rate of ATTRwt was unexpectedly high compared to other studies of cardiac amyloidosis where incidence of ATTRwt was reported to be 4-6%[3, 6]. The high incidence of ATTRwt in our cohort likely reflects the predominant cardiac presentation of ATTRwt, and the fact that our cohort were identified from cardiac biopsy samples. A recent study from the National Amyloidosis Centre (NAC) of the United Kingdom suggested ATTRwt

was clinically underrecognised prior to 2009[26]. The development of new non-invasive testing methods such as cardiac magnetic resonance imaging (cMRI) and bone scintigraphy were suggested to account for an increased cumulative incidence of ATTRwt diagnoses (25%) between 2015-2019 compared to 3% between 1987-2009. Consistent with previous studies, our patients with ATTRwt cardiac amyloidosis were predominantly male, older, had increased frequency of conduction abnormalities and did not have evidence of renal involvement[27]. However, these characteristics cannot be used to definitely subtype cardiac amyloidosis as there is significant overlap between ATTRwt and AL.

The advent of bone scintigraphy in recent years, enables a diagnosis of ATTRwt cardiac amyloid to be made in the absence of a tissue biopsy, but only in cases where there is no detectable plasma cell clone (i.e. no monoclonal protein on serum or urine EPG or immunofixation, and normal serum free light chain ratio). It is 99% sensitive and 86% specific for ATTRwt amyloid, however a proportion of patients with AL amyloid will have cardiac uptake on bone scintigraphy[28, 29]. Given that patients diagnosed with ATTRwt are usually older and the incidence of monoclonal gammopathy rises with age[30]; concurrent ATTRwt with evidence of plasma cell clonal gammopathy is not uncommon. In the instance of cardiac uptake on bone scintigraphy and a detectable plasma cell clone, accurate determination of amyloid subtype is essential to determine therapy and prognosis and can only be ascertained by cardiac biopsy and direct amyloid fibril assessment.

LMD-MS [12] is a diagnostic assay reported to be highly accurate for assessing the specific nature of the amyloid fibril protein. A number of reports have highlighted the potential

for amyloid subtype misdiagnosis using clinical phenotype or immunohistochemistry, without direct fibril assessment[7-9, 31]. Investigators from the NAC reported that of 350 patients thought to have AL, 10% were found to have amyloidogenic mutations and 24% of those patients were noted to have a concurrent low grade monoclonal gammopathy[31]. The limitations of relying on IHC for amyloid subtyping have been previously highlighted in an Australian cohort where IHC and LMD-MS were performed in 88 cases; in 49 cases IHC was non-diagnostic or uninterpretable, and in 5 of 39 cases the subtype was inaccurately characterised on the basis of IHC[11]. A further challenge to accurate diagnosis in the Australian setting is the lack of access to LMD-MS, as there is no current accredited local diagnostic laboratory and the cost of testing in overseas centres is unfunded. In our study, LMD-MS resulted in a definitive subtype for 39 patients and clarified the diagnosis in 3 complex patients. While misdiagnosis appears to only occur in a small percentage of patients, and has probably been reduced using new diagnostic algorithms (see figure 3) incorporating bone scintigraphy, substantial differences in prognosis and treatment highlight the importance of an accurate diagnosis[3, 6, 32, 33].

The limitations of this study arise from the small number of participants, and the retrospective nature of the data. The study context also deserves consideration as St Vincent's hospital is a quaternary referral centre for cardiac transplantation, with expertise in endomyocardial biopsy. This referral bias may have impacted the presentation of amyloid subtypes in the study. However, given the rarity of this disease, these limitations are offset by the opportunity to gain insights from a cohort of patients with long follow up and biopsy-proven cardiac amyloidosis. It would be valuable to

confirm these findings with data from large prospective studies and registries in light of novel therapies for AL and ATTRwt.

Conclusion

This study fills an important gap in the epidemiology of cardiac amyloid in Australia and reports survival outcomes on the basis of highly accurate amyloid fibril assessment. AL amyloid was associated with very poor survival, predicted on the basis cardiac failure symptoms and bone marrow plasmacytosis. TTR amyloidosis was more common than previously described, affecting almost a third of patients, however the majority of these patients could not be offered any specific therapy at the time, and over half of patients died within 5 years. There are a number of new treatments now available or undergoing investigation including TTR stabilisers (diflunisal, tafamidis) and TTR synthesis inhibitors (patisiran and inotersen)[33], although none are currently funded in Australia. Despite the increasing emergence of diagnostic strategies (see Figure 3) that may abrogate the need for tissue biopsy in some cases, LD-MS plays an important role in complex cases. The establishment of the Australian Amyloidosis Network facilitating collection and publication of data in this rare patient group will hopefully lead to earlier and more accurate diagnosis and optimised management pathways.

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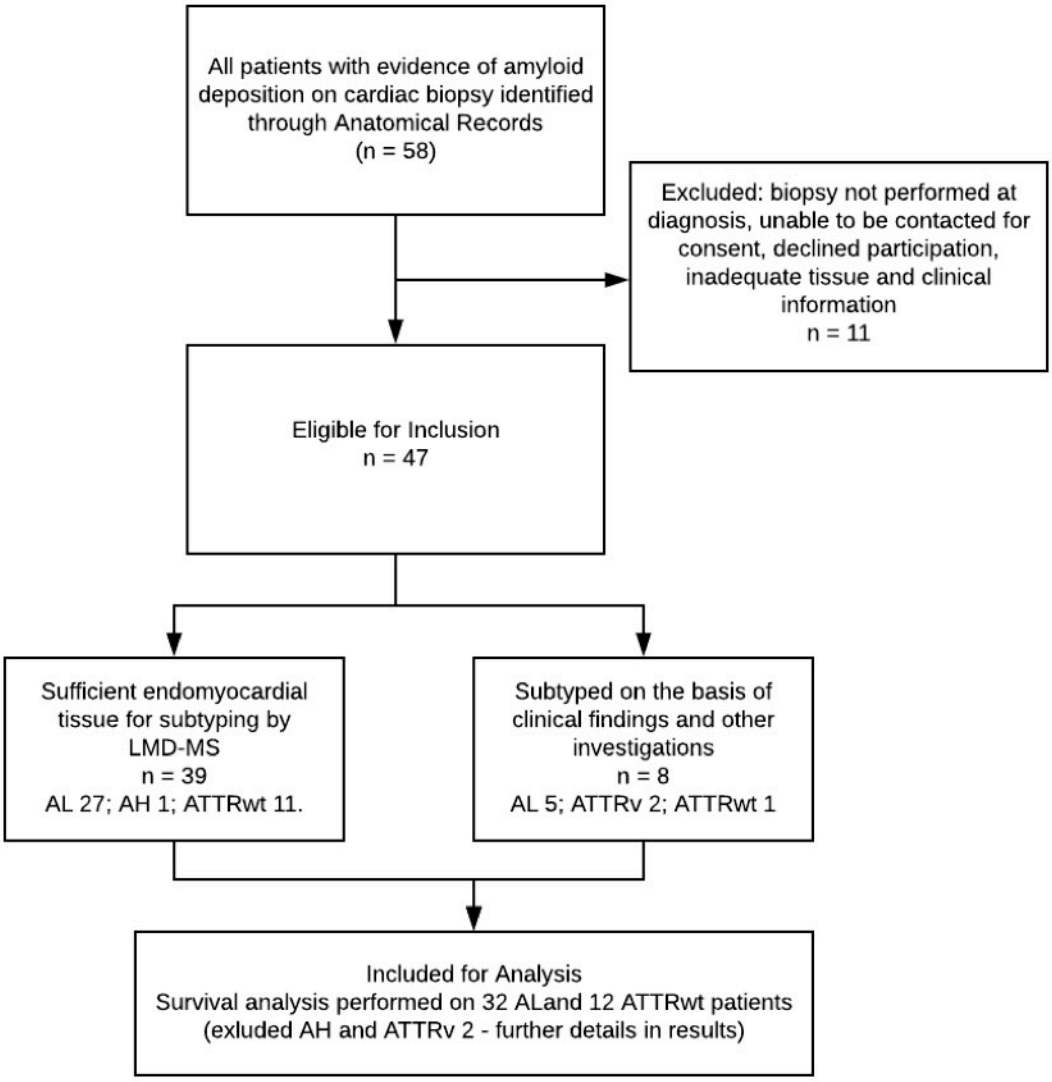
Table 1. Baseline clinical characteristics

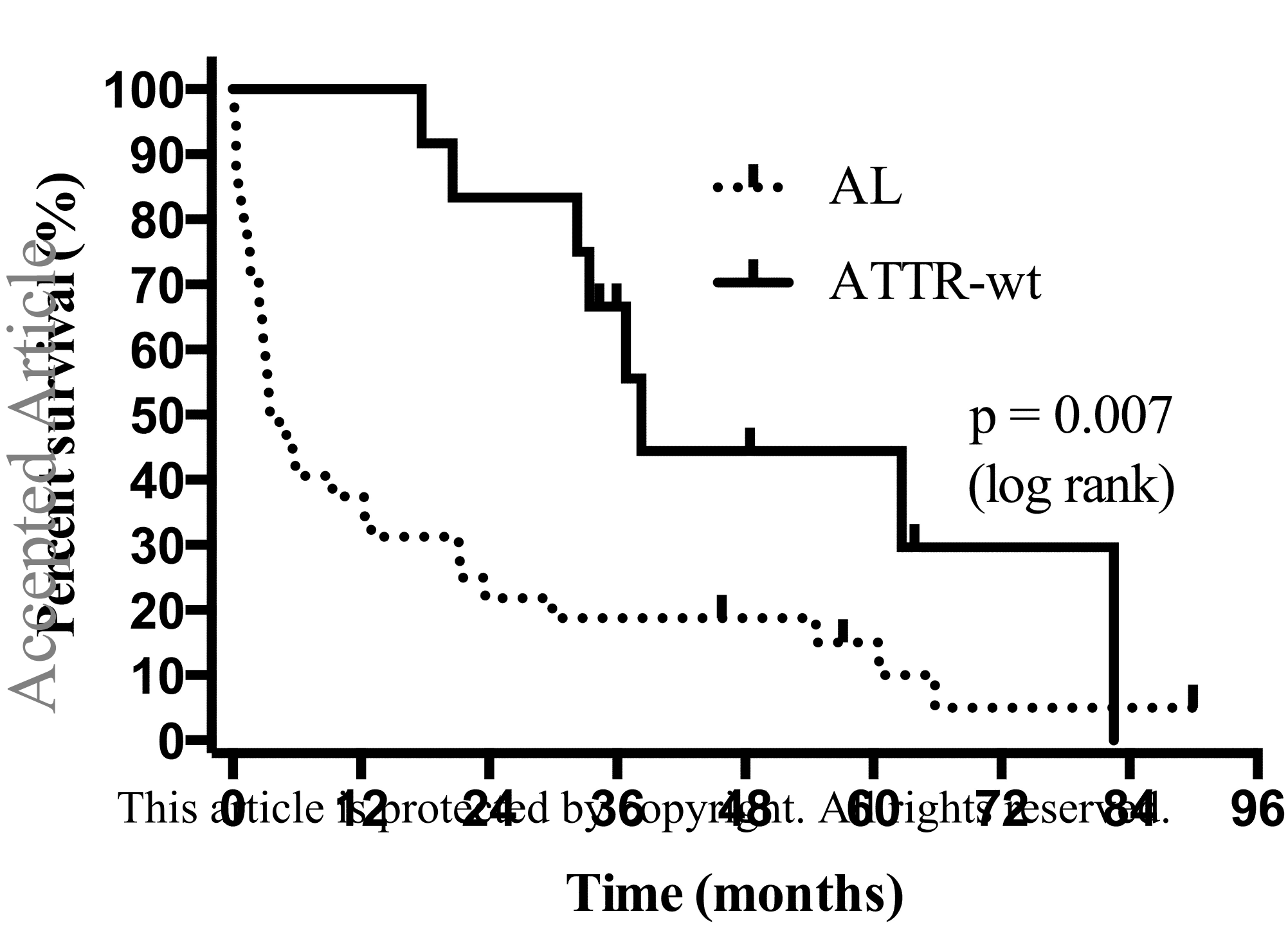
Table 2. Cox regression survival analysis of diagnostic variables in AL

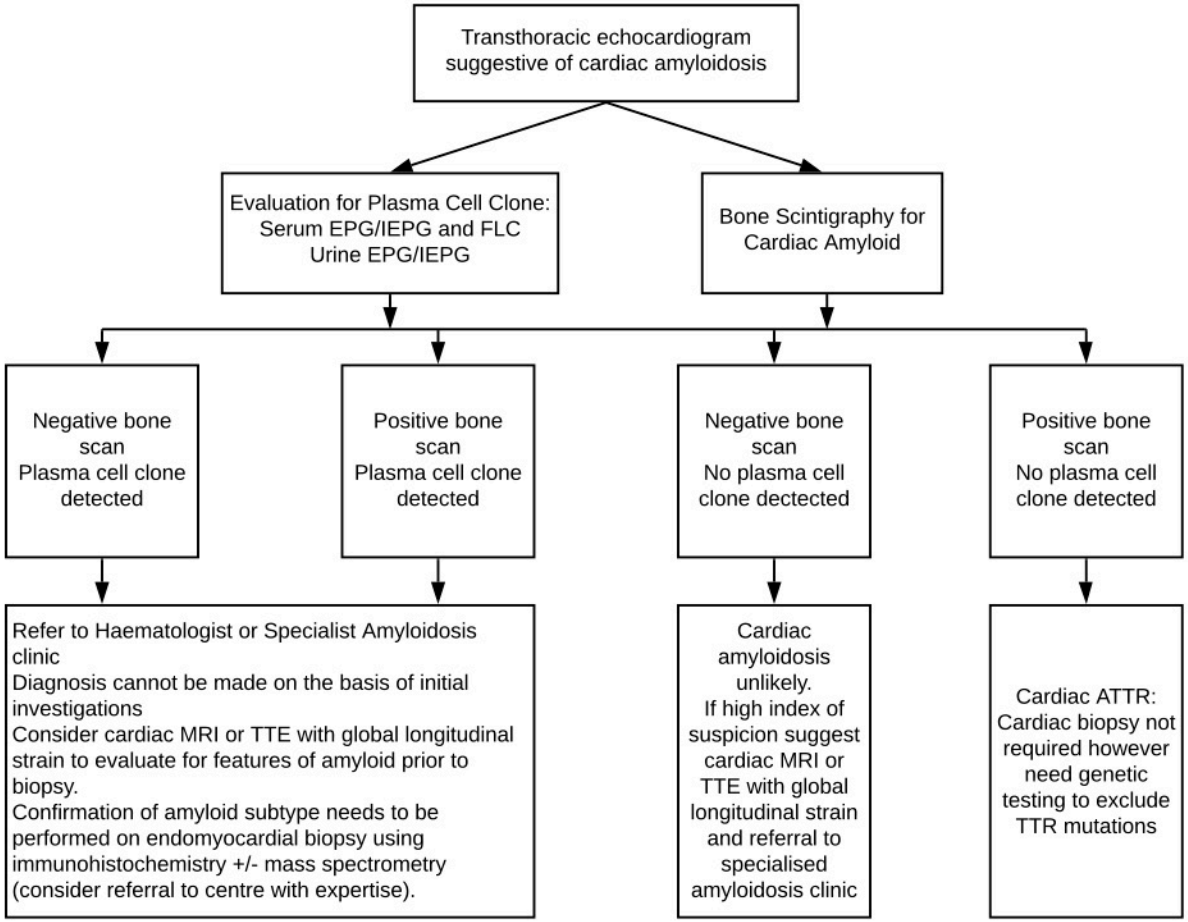
Figure 1. Study flow chart

Figure 2. Kaplan-Meier survival curve of ATTRwt vs. AL showing significant poorer survival of AL patients

Figure 3. Diagnostic approach to suspected cardiac amyloidosis







Characteristic (n= evaluable patients in each group†)	**ATTRwt (n=12)**	**AL/AH (n=33)**	**ATTRv (n=2)**	**P value‡**
Male sex, n (%)	11 (92)	15 (46)	2(100)	**0.006**
Age at diagnosis, y - median (range),	76 (69-88)	60 (41-83)	52 (49-55)	**< 0.001**
Symptom duration, m - median (range), (n=9/26/2)	18(4-84)	10 (1-48)	34 (7-60)	0.093
Symptoms, n (%) (n=9/26/2)				
Dyspnoea on exertion	8 (89)	25 (96)	2 (100)	0.454
Dyspnoea at rest	2 (22)	7 (27)	0	1.000
Syncope	1 (11)	4 (15)	1 (50)	1.000
Dizziness	0	7 (27)	1 (50)	0.153
Palpitations	1 (11)	5 (19)	1 (50)	1.000
Peripheral oedema	6 (67)	17 (65)	1 (50)	1.000
Effusions or ascites	3 (33)	10 (39)	0	1.000
Upper GI symptoms	5 (56)	16 (62)	0	1.000
Lower GI symptoms	0	8 (31)	1 (50)	0.081
Weight loss	2 (22)	14 (54)	1 (50)	0.135
Organ involvement, n (%)				
Renal (n=5/23/2)	0	14 (61)	0	**0.041**
Liver (n=7/25/1)	2 (29)	8 (32)	0	1.000
Carpal tunnel symptoms/surgery (n=12/29/2)	5 (42)	10 (35)	1 (50)	0.730
Gastrointestinal (on biopsy)	0	3	1	
Peripheral nerve (symptomatic)	0	4	1	
Macroglossia (symptomatic)	0	3	0	
Other (see text)	0	3	0	
>2 organs involvment (including cardiac) (n=8/28/2)	1 (13)	12 (43)	1 (50)	0.213
Baseline laboratory values				
Monoclonal protein in serum, g/L, n (%) (n=9/26/2)	0	14 (54)	0	**0.005**
Monoclonal protein in urine, g/L, n (%) (n= 8/26/2)	0	17 (65)	0	**0.001**
Abnormal free light chain ratio, n (%) (n= 6/19/1)	0	18 (95)	0	**<0.001**
Median κ light chain, mg/L (range) (n=6/18/1)	17 (8-25)	22 (5-4470)	15	0.626
Median λ light chain, mg/L (range) (n=6/19/1)	21(11-44)	209 (7-2524)	19	**0.043**
Median light chain difference, mg/L (range) (n=17)	6 (1-23)	455 (68-4462)	4	**<0.001**
Plasma cells >10% on bone marrow aspirate, n (%) (n=2/20/2)	0	7 (35)	0	1.000
Median BM plasma cell percentage, % (range) (n=1/16/1)	1	7 (2-50)	2	0.118
Proteinuria >0.5g/d, n % (n=5/23/2)	0	13 (57)	0	**0.044**
Median urine protein, g/24hr (range) (n=3/18/1)	0.19 (0.18-0.32)	1.26 (0.07-4.98)	0.17	0.307
Renal impairment, n (%), (n=9/27/2)	1(11)	13 (48)	0	0.062
Cardiac Parameters				
NYHA Class I-II, n (%) (n=11/28/2)	8 (73)	17 (61)	2 (100)	0.713
Prior cardiac arrhthymia or pacemaker insertion (n=12/29/2)	9 (75)	9 (31)	1 (50)	**0.016**
ECG - low voltage, n (%) (n=8/22/2)	4 (50)	17 (77)	0	0.195
EF < 50% - systolic dysfunction, n (%) (n=9/26/1)	4 (44)	6 (23)	0	0.393
Median interventricular septal thickness, mm (range) (n=7/25/2)	22.0 (12-24)	15.0 (10-21)	16.5 (15-18)	**0.005**
Raised NT-proBNP above reference range, n (%) (n=5/12/1)	5 (100)	12 (100)	1 (100)	
NT-proBNP, ng/L (range) (n=5/12/1)	1931 (855-8810)	4781 (415-34340)	1350	0.506
Raised troponin I/T above reference range, n (%) (n=4/17/1)	3 (75)	11 (65)	0	1.000
Median troponin I, ug/L (range) (n=0/6/0)	-	0.225 (0.030-0.430)	-	
Median troponin T, ng/L (range) (n=4/10/1)	67 (30-82)	64 (10-486)	10	1.000

†Sample size as stated for each subtype unless otherwise indicated.

‡ P value refers to comparison between ATTRwt and AL/AH groups only.

Independent Variable	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Age >= 60 yrs	1.67 (0.77-3.66)	0.2		
Male	0.69 (0.33-1.44)	0.3		
Symptoms prior to diagnosis >= 12 mth	1.29 (0.57-2.94)	0.5		
Organs involvement >=3	0.60 (0.26-1.35)	0.2		
Proteinuria >=0.5g/d	0.58 (0.23-1.45)	0.2		
Renal Impairment, Cr females>=90umol/L, males>=120umol/L	**2.48 (1.01-6.09)**	**0.05**		
Year of diagnosis prior to 2000	**2.76 (1.22-6.24)**	**0.02**		
NYHA severity stage III/IV	**5.02 (1.78-14.13)**	**0.002**	**5.21 (1.21 – 22.49)**	**0.03**
Interventricular septal width >=16mm	0.94 (0.39-2.28)	0.9		
Systolic dysfunction EF < 50%	0.98 (0.36-2.70)	0.97		
Troponin I >=0.1ug/L or T >=77ng/L	**4.72 (1.33-16.69)**	**0.02**		
NT-proBNP >=2736ng/L	1.97 (0.49-7.86)	0.3		
Kappa light chain restriction	1.51 (0.63-3.62)	0.4		
Lambda light chain restriction	0.66 (0.28-1.60)	0.4		
iFLC κ >=900mg/L or λ >=300mg/L	2.01 (0.71-5.72)	0.2		
dFLC >=180mg/L	2.27(0.50-10.27)	0.3		
dFLC >=450mg/L	3.11(0.91-10.60)	0.07		
Plasma cells on marrow aspirate >=10%	**3.88 (1.28-11.75)**	**0.02**	**3.26 (0.97-10.91)**	**0.05**