Prognostic Markers and Management Strategies in Systemic Amyloidosis

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Declaration

I, Oliver Charles Cohen, confirm that the work presented in this thesis is my own. I have acknowledged work derived from other sources.

Abstract

Background

Systemic amyloidosis is increasing in prevalence but remains a rare, potentially fatal disorder characterised by the misfolding of autologous proteins into an abnormal fibrillar form. Outcomes are dependent on amyloid subtype.

Aims

To improve diagnosis, management and understanding of prognosis of patients with amyloidosis. In terms of diagnosis, I will evaluate the diagnostic sensitivity of screening biopsies in AL and ATTR amyloidosis. I will also assess prognostic markers at baseline, and following treatment, in a large UK cohort of uniformly treated patients with a view to developing a new staging system for AL amyloidosis incorporating novel prognostic markers. I will focus on functional markers of prognosis such as longitudinal strain on echocardiogram and the 6-minute walk test. I shall also explore treatment outcomes with novel therapies (ixazomib-lenalidomide-dexamethasone and daratumumab) in AL amyloidosis and the impact of chemotherapy on quality of life in patients with AL amyloidosis at a variety of timepoints. Finally, I will assess the value and outcomes of organ transplantation in patients with AL amyloidosis and apolipoprotein A-I amyloidosis.

Results and Conclusion

Screening biopsies are valuable, particularly in AL amyloidosis, to avoid higher-risk target organ biopsies. Longitudinal strain, measured via echocardiogram, is prognostic at baseline and following chemotherapy in AL amyloidosis. It is

independent of Mayo criteria and can be incorporated into a new prognostic staging system alongside cardiac biomarkers both at baseline and in evaluating response to treatment. The 6-minute walk test distance is also prognostic at baseline, whilst improvement in walk test at 12 months predicts survival. Health-related quality of life improves in patients achieving a complete haematological response or cardiac organ response and is an important marker in the holistic characterisation of treatment response. Ixazomib-lenalidomide-dexamethasone and Daratumumab monotherapy are both effective treatment options in AL amyloidosis. Renal transplantation is associated with very encouraging outcomes in carefully selected patients with AL amyloidosis. In Apolipoprotein A-I amyloidosis, renal, hepatic and cardiac transplantations all have a role and are associated with good long term outcomes.

Impact Statement

The content of this thesis is aimed at improving diagnosis, prognostication and treatment of amyloidosis with a primary focus on AL amyloidosis. It explores a variety of functional prognostic markers in AL amyloidosis as well as novel treatments for use in the relapse setting. The work described here has led to a number of publications and international oral presentations, which has improved awareness of the diagnosis, prognosis and management of this rare disease.

An exploration of the diagnostic sensitivity of screening biopsies has demonstrated that target-organ biopsies are only required in a relatively small proportion of patients with systemic AL amyloidosis, which may reduce the frequency of clinicians organising upfront target organ biopsies, which are more invasive and costly.

This work reports the value of longitudinal strain by echocardiogram in the largest cohort of patients with cardiac AL amyloidosis reported to date. Current prognostic systems rely solely on cardiac biomarkers, which are non-specific and variable. Longitudinal strain is independent of traditional cardiac biomarkers and, here, a new staging system is proposed, for use at baseline and following treatment with chemotherapy, which is independent of traditional biomarker-based staging with the aim of providing patients and clinicians with more accurate prognostic information. Similarly, the value of the 6-minute walk test is evaluated at baseline and following therapy, and is found to be independent of Mayo stage criteria thus also providing additional prognostic information, which may guide treatment decisions and better inform clinicians and patients.

The impact of health-related quality of life in AL amyloidosis is sparsely reported. The work presented in this thesis characterises the impact of cytotoxic treatment, haematological and organ response on patient-reported quality of life. This essential aspect of characterising treatment response can aid clinician understanding of the impact of amyloid-directed treatment on patients and further highlights the value of obtaining a deep haematological response in the AL amyloidosis setting.

Improved awareness of amyloidosis, leading to more prompt diagnosis, and increasingly effective upfront treatment regimens have led to improved survival such that patients are living through multiple relapses of their disease thus the need for new novel therapies and treatment combinations is increasingly essential. This thesis examines treatment outcome with two novel therapies, specifically ixazomib-lenalidomide-dexamethasone and daratumumab monotherapy. The combination of ixazomib-lenalidomide-dexamethasone in this setting has not previously been reported. Both combinations are tolerable and can induce deep haematological response in multiply relapsed patients with AL amyloidosis.

Finally, as patient survival improves, greater consideration must be given to the value of organ transplantation in amyloidosis. Amyloidosis can lead to devastating organ dysfunction inclusive of renal failure and yet these same patients can potentially survive long term after achieving excellent haematological responses to therapy. Consequently consideration of organ transplantation to improve quality of life should be increasingly considered. Based upon our cohort of fifty patients with AL amyloidosis with renal involvement, renal transplantation leads to good outcomes in carefully selected patients. At relapse, the renal grafts were not devastatingly impacted by further therapy and graft failure due to recurrence of amyloid was rare at

long-term follow up. Equally, encouraging transplant outcomes were seen in a population of patients with Apolipoprotein A-I amyloidosis. The natural history of a large cohort of fifty-seven patients with Apolipoprotein A-I amyloidosis is reported with a focus on the eighteen patients who underwent organ transplantation. Of note, patients who underwent liver transplantation demonstrated regression of amyloid in other visceral organs over time.

Ethical Approval

Explicit written consent was obtained from all patients whose data was used in the clinical research studies described below in the form of a signed consent form obtained during their initial visit to the National Amyloidosis Centre. The consent form was approved by the Royal Free Hospital Ethics Committee (REC Ref 06/Q0501/42). The dosage and administration of radioactive isotope used for ^{123}I-serum amyloid-P component scintigraphy were approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health.

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I would like to thank my wife, Rachel, for her encouragement and never-ending patience, which has allowed me to pursue my chosen career and research interests. I am thankful to my sons for providing my inspiration to improve myself and to my parents and wider family for their unconditional support.

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Abbreviations

Six minute walk distance	6MWD
Six minute walk test	6MWT
Systemic amyloid A	AA
Adverse event	AE
Abdominal fat aspirate	AFA
Immunoglobulin light chain	AL
Apolipoprotein A-I	APOAI
Autologous stem cell transplantation	ASCT
Transthyretin amyloidosis	ATTR
Beta-2 microglobulin	ß2M
Bodily pain	BP
Bone marrow trephine	BMT
Confidence Interval	CI
Chronic kidney disease	CKD
Cardiac magnetic resonance imaging	CMR
(R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid	CPHPC
Complete haematological response	CR
Creatinine clearance	CrCl
Common Terminology Criteria for Adverse Events	CTCAE
Cyclophosphamide-Bortezomib-Dexamethasone	CyBorD
Difference between involved and uninvolved light chains	dFLC
Eastern Cooperative Oncology Group performance status	ECOG
Extracellular volume	ECV
Estimated glomerular filtration rate	eGFR

End-stage renal failure	ESRF
Free light chain	FLC
Gastrointestinal	GI
General Health Perception	GH
Health-related quality of life	HRQL
Heart Failure with preserved ejection fraction	HFpEF
Hereditary transthyretin amyloidosis	hATTR
High-density lipoprotein	HDL
Involved free light chain	iFLC
Immunomodulatory drug	IMiD
Ixazomib-lenalidomide-dexamethasone	IRd
^{123}Iodine-labelled serum amyloid P component scintigraphy	^{123}I-SAP
Late gadolinium enhancement	LGE
Longitudinal strain	LS%
Left ventricle	LV
Mental Component Summary	MCS
Mental Health	MH
Minimal residual disease	MRD
National Amyloidosis Centre	NAC
Next generation sequencing	NGS
N-terminal probrain natriuretic peptide	NT-proBNP
No response to therapy	NR
New York Heart Association class	NYHA
Overall survival	OS
Physical Component Summary	PCS
Physical Functioning	PF
Positron emission tomography	PET
Positron emission tomography – computed tomography	PET-CT

Progression-free survival	PFS
Partial response	PR
Quality of Life	QoL
Ribonucleic acid	RNA
Renal replacement therapy	RRT
Role-Emotional	RE
Role-Physical	RP
Serum amyloid A protein	SAA
Serum amyloid P component	SAP
Serum free light chain	sFLC
Social Functioning	SF
Steady-state free precession	SSPE
^{99m}Technetium-3,3-diphospono-1,2-propanodicarboxylic -acid scintigraphy	^{99m}Tc-DPD
^{99m}Technetium-labeled pyrophosphate	^{99m}Tc-PYP
Time to next treatment	TNT
Very good partial response	VGPR
Vitality	VT
Wild-type transthyretin amyloidosis	wtATTR

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Chapter One: Introduction

This chapter is written in the context of my publication:

Systemic Amyloidosis: Moving into the spotlight. Cohen OC & Wechalekar AD. Leukemia (2020), 34, 1215-1228. Copyright permission obtained from Leukemia office for use in my thesis.

Systemic amyloidosis is a rare but increasingly recognised disease that is heterogeneous in presentation. Autologous proteins misfold and aggregate into an abnormal fibrillar form, which deposit in tissues leading to progressive dysfunction (1). Whilst still considered a rare disease, the number of cases of amyloidosis seen at the UK NAC increased by 670% from the period 1987-1999 to the period 2010-2019 (2). The amyloid subtype is classified based upon the involved fibril protein, of which 35 have been identified (3). An epidemiological study in the United States of America reported a doubling in prevalence of AL amyloidosis from 15.5 cases per million in 2007 to 40.5 cases per million in 2015 despite a stable incidence (4) reflecting key advances in detection, response assessment and the availability of novel agents. Whilst AL amyloidosis predominates, the recognition of wtATTR amyloidosis is rapidly increasing. Conversely, the declining prevalence of AA amyloidosis reflects key advances in the control of inflammatory pathologies such as rheumatoid arthritis and inflammatory bowel disease.

Fibrillogenesis, Deposition and Degradation of Amyloid Proteins

The mechanisms involved in fibrillogenesis remain poorly understood. Despite the heterogeneity of the involved precursor proteins, the structure of the resultant amyloid fibril is highly consistent - an insoluble twisted ß -pleated sheet polypeptide. Amyloid fibrils are visualised by electron microscopy as unbranched, 6-20 nanometre wide rope-like polymers that can be several micrometres in length (5). When stained with Congo red dye, these polypeptides classically exhibit apple-green birefringence under cross polarised light. Amyloid deposits also contain non-fibrillar components including serum amyloid P component, apolipoprotein E and other proteins such as proteoglycans and glycosaminoglycans (6). It is the binding of SAP to the amyloid fibril that prevents proteolysis thus contributing to fibril persistence in vivo (7).

Amyloid fibril deposition was initially described in the context of disease yet functional amyloid proteins with normal biological activities also occur (8). In systemic AL amyloidosis, the amyloidogenic protein is abnormal, produced by a clonal population in bone marrow. An abnormal protein variant is also responsible for hereditary amyloid subtypes including hATTR, apolipoprotein A-I, apolipoprotein A-II, apolipoprotein A-IV, Fibrinogen, lysozyme and Gelsolin amyloidosis. In contrast, other subtypes are associated with an excess of a normal protein as in the case of AA amyloid, due to an increase in SAA in chronic inflammatory states and ß2M in dialysis-related amyloidosis.

Amyloidosis is characterised by progressive dysfunction of the heart, liver and kidneys, which interplays with variable damage to nerves, soft tissue and the gastrointestinal tract. However, the pattern of organ involvement is dependent upon the amyloid fibril in question. Amyloid-related tissue damage results, in part, from direct tissue disruption whilst further mechanisms may be specific to amyloid subtype.

In multiple myeloma, high levels of circulating light chains are relatively common but not necessarily amyloidogenic. In AL amyloidosis, the initiation of amyloid formation could occur secondary to factors including high-levels of light chain expression, the sequence or post-translational modification of individual light chains or by light chain fragmentation (9). Amyloidogenic light chain immunoglobulins exhibit direct proteotoxic activity. In cardiac myocytes, fibroblasts internalise the amyloidogenic light chains, which leads to alterations in proteoglycan structure and resultant tissue damage (10). In contrast, pre-fibrillar oligomers are thought to represent the primary pathological subunit in both Alzheimer's disease and ATTR amyloidosis (11). The relative contribution of organ damage secondary to tissue disruption and that due to fibril toxicity may differ between amyloid subtypes.

Resorption of amyloid deposits may occur if the aetiological mechanism underlying their deposition ceases. In AL amyloidosis, chemotherapy targeting the underlying plasma cell clone in bone marrow reduces the level of amyloidogenic FLC whilst in AA amyloidosis, targeting the underlying inflammatory state reduces further amyloid deposition. In ATTR amyloidosis, transthyretin production occurs in the liver thus, historically, orthotopic liver transplantation (12) and, more recently, RNA silencers such as patisiran (13) and inotersen (14), have been implemented as disease-modifying therapies to inhibit hepatic synthesis of transthyretin. The mechanisms underlying amyloid resorption are poorly understood but thought to be macrophage-driven (15). A growing body of evidence suggests that infiltrating innate immune cells may play key roles in clearing cerebral amyloid-ß plaques in Alzheimer's disease (16). Furthermore, murine models have demonstrated that the administration of anti-human SAP antibodies trigger a macrophage-derived giant cell reaction, which

is complement-dependent and results in clearance of the deposit in question. Key subtypes of amyloidosis are listed in **Table 1.1**.

Table 1.1: Key Subtypes of Amyloidosis

Subtype	**Prevalence**	**Precursor Protein**	**Common Organ Involvement**	**Mainstay of Treatment**
AL	68%	Monoclonal light chain	Cardiac, renal, liver, peripheral nervous system, autonomic nervous system, gastrointestinal and soft tissue	Systemic chemotherapy and monoclonal antibodies directed at the underlying bone marrow clone
ATTR:				
Hereditary:	6.6%	Variant-dependent	Cardiac, peripheral nervous system, autonomic nervous system	Transthyretin stablisisers (Diflunisal, Tafamidis) Gene-silencing therapy (Inotersen, Patisaran)
Wild-type:	3.2%	Wild-type transthyretin	Cardiac, carpal tunnel syndrome	Supportive or clinical trial
AA	12%	Serum amyloid A	Renal, liver, gastrointestinal	Reduction in SAA via corticosteroid, cytostatic drugs and monoclonal antibodies e.g. anti-tumour necrosis factor, anti-interleukin-6.
Fibrinogen	1.7%	Fibrinogen alpha-chain	Renal, liver	Supportive, renal transplant
Apolipoprotein A-1	0.8%	Apolipoprotein A-1	renal, liver, cardiac, larynx, skin, testes.	Supportive

Types of Amyloidosis

Systemic AL amyloidosis

Systemic AL amyloidosis remains the most common form of the disease accounting for 55% of cases (2) with a yearly incidence of approximately 12.5 cases per million (17). It is characterised by the production of an amyloidogenic light chain by a plasma (or other B) cell population in bone marrow. Systemic AL amyloidosis most commonly causes damage to the heart and/or kidneys in 60-75% and 50-70% of cases respectively (18) with variable involvement of other organs including the liver, gut, nervous system and soft tissues. Peri-orbital bruising, indicative of soft tissue involvement, is almost pathognomonic of AL amyloidosis.

Cardiac involvement predicts prognosis with a median survival, if untreated, of 6 months from the onset of heart failure (19). Consequently, cardiac biomarkers underlie the Mayo staging system, which uses NT-proBNP and troponin to predict prognosis (20). This staging system has since been modified to include baseline dFLC (21). Most recently, a European collaborative study identified that NT-proBNP >8500ng/L predicted survival independently and defined a new subgroup within the original Mayo stage III (2004) with a particularly poor prognosis of just 3 months (22).

Chemotherapy remains the mainstay of treatment in systemic AL amyloidosis. The advent of novel therapies together with improvements in supportive case and selection for autologous transplantation have led to marked improvements in prognosis over time. A large American study demonstrated that 4-year overall survival improved in both transplant eligible and ineligible patients between the periods 2000-2004 and 2010-2014 (ASCT eligible: 65% to 91%; ASCT ineligible: 16% to 38%). Furthermore, patients were less likely to die with 6 months of diagnosis (24% vs. 37%) (23).

Localised AL amyloidosis

Localised deposits of AL amyloidosis, caused by a localised clonal dyscrasia within the affected tissue, can occur almost anywhere. It is rare compared to systemic AL amyloidosis. Common sites include the urinary tract (16%), larynx (15%), skin (14%) and respiratory tract (8%). Management is supportive and dependent upon local issues occurring secondary to the presence of the amyloid deposit. This commonly involves local excision of troublesome deposits. It is very rare that patients with localised disease and no detectable clone in bone marrow go on to develop systemic disease and, as such, prognosis is excellent with no apparent effect on life expectancy (24).

Wild-type transthyretin amyloidosis

The recognition of wild-type transthyretin amyloidosis is increasing and has risen from <3% in the period 1987-2009 to 25% in the last 4 years (2). A study of patients with HFpEF suggested that 13% may have underlying wtATTR amyloidosis (25). It is also termed senile systemic amyloidosis as it is typically a disease of older people. It is slowly progressive and characterised by cardiac failure although deposits are also found in other tissues with high mechanical stress such as the lungs, bladder and vascular walls (26). However, extra-cardiac deposits are commonly asymptomatic. It is the overproduction of normal transthyretin, by the liver, that form the basis of the amyloid fibril (27). Whilst the management remains largely supportive, the European Commission has recently approved the use of Tafamidis, a selective transthyretin stabiliser, which has been shown to reduce all-cause mortality, functional decline and cardiovascular-related hospital admissions compared to placebo (28). In

the near future, wild-type ATTR amyloidosis may well emerge as a major public health issue in the elderly.

Hereditary transthyretin amyloidosis

Over 100 genetic variants at hATTR amyloidosis have been documented since it was first described in Portugal in 1952 (29). The prevalence of these subtypes vary geographically and are inherited in an autosomal dominant fashion with variable penetrance. The commonest variant worldwide is characterised by a substitution of methionine for valine at position 30 (V30M) and is most common in patients with Portuguese ancestry. A report of hATTR amyloidosis cases seen at the National Amyloidosis centre, United Kingdom, found that the two commonest subtypes diagnosed were the p.V142I and p.T80A variants predominantly diagnosed in African/Caribbean and Irish patients respectively (30). The clinical phenotype also varies between subtypes but is dominated by progressive cardiac failure and/or peripheral and autonomic neuropathy. As in wtATTR, the amyloid fibril consists of transthyretin. The natural life expectancy is 9 to 13 years post-symptomatic presentation and death usually results from cardiac involvement or cachexia (31).

Historically, management was supportive or with orthotopic liver transplantation. This procedure does not usually reverse neuropathy or organ impairment but does typically halts progression. The 5 year survival is 100% in patients with V30M and 59% for other variants (12). More recently, RNA silencers (Patisiran (13), Inotersen (14)) have been approved for patients with neurological involvement and have been shown to improve multiple clinical manifestations of the disease. Clinical trials are underway to investigate the use of similar agents in participants with Transthyretin-mediated amyloid cardiomyopathy (e.g. Eplontersen [NCT04136171]).

Systemic AA amyloidosis

Systemic AA amyloidosis is characterised by the deposition of amyloid fibrils, derived from serum amyloid A protein. The SAA protein is produced in the liver in high concentrations in the context of underlying inflammatory states. The prevalence is decreasing with the advent of effective anti-inflammatory therapies to treat such conditions. A recent systematic review documented 48 conditions strongly associated with the development of AA amyloidosis, which were broadly grouped under the headings of chronic infection, chronic inflammatory states, hereditary syndromes and malignancy (32). AA amyloidosis commonly deposits in the kidney but may also involve the liver and gut. Asymptomatic splenic involvement is common and can be visualised on ^{123}I-SAP scintigraphy. Management is directed towards the underlying cause and aims to suppress the SAA level to prevent ongoing amyloid fibril deposition. At follow up, patients with low SAA values are more likely to demonstrate regression of amyloid deposits on ^{123}I-SAP scintigraphy (33).

Hereditary fibrinogen Aα-chain amyloidosis

Hereditary fibrinogen amyloidosis is inherited in an autosomal dominant fashion and was initially characterised in Peruvian kindred in 1993 (34). A number of variants have since been described with E526V, mainly reported in Northern Europeans, the most common (35). This form of amyloidosis predominantly affected the kidneys, presenting with proteinuria, hypertension or chronic kidney disease. Patients present at a median age of 58 years with a median time from presentation with proteinuria to ESRF of 4.6 (range 0-10.2) years (36). The mainstay of management is supportive with renal transplantation an option in selected patients. Isolated renal transplantation is associated with excellent graft survival (68% at 10 years) despite a median time to

amyloid deposition in the transplanted kidney (based on ^{123}I-SAP scintigraphy) of 7 years (37).

Apolipoprotein A-I amyloidosis

Apolipoprotein A-I is 28-Kda, non-glycosylated and the main apolipoprotein of HDL (38). Eighteen different amyloidogenic APOAI variants with resultant slowly progressive organ dysfunction are reported. Renal (39-46), cardiac (42, 47-51) and hepatic (39, 41, 42, 46, 52) involvement occurs in 11, 6 and 5 variants respectively. Further variants have been associated with localised deposits in the aortic intima (with associated angina) (53) and palate (40) without co-existent organ dysfunction. Patients with renal involvement secondary to APOAI amyloidosis present with slowly progressive chronic kidney disease, hypertension or proteinuria. Hepatic involvement typically leads to hepatomegaly due to infiltration by amyloid but liver failure, usually by the 6th decade, is reported in a Spanish family with a Leu60Phe71 deletion / insertion Val60Thr61 variant within the APOAI gene. Within the heart, amyloid deposits lead to a progressive restrictive cardiomyopathy manifesting as congestive cardiac failure (48). Other recognised manifestations include infertility (40, 49), hoarse voice/dysphonia (40, 48, 50, 51), polyneuropathy (39, 40, 45, 49) and cutaneous lesions (47, 48).

The mainstay of management in APOAI amyloidosis is supportive. A series of 14 patients with APOAI amyloidosis undergoing renal transplantation documented a median graft survival of 13.1 years, which was notably longer than graft survival in patients with AL (12.4 years), AA (10.3 years) or Fibrinogen-type (7.3 years) amyloidosis (54, 55).

Recognition of amyloidosis

Early symptoms of amyloidosis are non-specific (e.g. peripheral oedema, dyspnoea), which commonly poses a diagnostic challenge and may delay diagnosis. The patient pathway to diagnosis is variable. Most patients see a median of 4 specialists prior to diagnosis with one-third of patients experiencing delays of ≥1 year from symptom onset (56). This pathway is dominated by haematology, nephrology and cardiology but other common clinical manifestations include neuropathy, macroglossia and a bleeding diathesis.

There are specific clues, which may guide diagnosis, in each of the relevant specialties. In the haematology clinic, all patients with systemic AL amyloidosis have an underlying clonal dyscrasia. Critically, a seminal study has demonstrated that a monoclonal immunoglobulin was detected in all samples taken ≤4 years before diagnosis (57) presenting a clear window of opportunity to identify amyloidosis early via vigilant assessment. In AL amyloidosis, 98% of cases are associated with an abnormality of the FLC ratio. In the cardiology clinic, patients may present with heart failure with preserved ejection fraction, which should prompt further investigation to exclude amyloidosis, particularly if associated with a disproportionately high NT-proBNP. Carpal tunnel syndrome provides a key clue to diagnosis in all clinical contexts. Patients with renal involvement are often diagnosed after referral to nephrology following an incidental finding of proteinuria in primary care. At a later stage, these patients may present with symptomatic peripheral oedema secondary to nephrotic syndrome. Systemic AA amyloidosis should be considered in the presence of an underlying chronic inflammatory state. A careful family history is paramount, particularly in cases of renal, cardiac or neuropathic involvement to exclude hereditary forms of the disease.

Diagnosis of amyloidosis

The diagnosis of amyloidosis is made by demonstrating amyloid deposition histologically or via a highly-specific diagnostic imaging modality. Histological diagnosis has the advantage of allowing for subtyping of amyloid deposits in addition to confirmation of diagnosis.

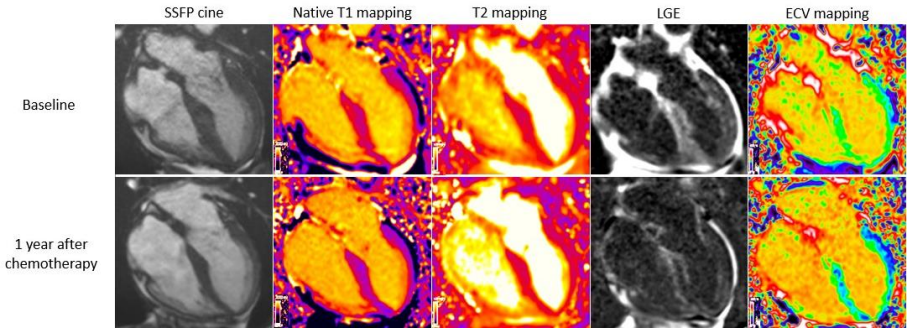
Histological diagnosis of amyloidosis

Congo red staining, exhibiting characteristic birefringence under cross polarised light, remains the gold standard. Whilst the biopsy of an affected organ has the highest yield, it carries a bleeding risk. Screening biopsies, inclusive of abdominal fat aspiration, are a preferred low-risk alternative, which detect amyloid in over 3/4 of cases of cardiac AL amyloidosis (58). The cost and complexity of typing amyloid fibrils are major deterrents for regional hospitals. Furthermore, it is debateable whether typing is essential in all cases. In cases of a clear free light chain excess, soft tissue amyloid (a pathognomonic feature of AL amyloidosis) and multi-organ involvement, typing could conceivably be omitted. Conversely, typing is critically important in cases of isolated renal or cardiac involvement to exclude non-AL amyloidosis. The use of laser microdissection and capture of Congo red positive tissue followed by protein identification by mass spectrometry and bioinformatics, greatly improves sensitivity and specificity of amyloid fibril typing (59). This approach is strongly recommended unless there is a well-established program of routine amyloid immunohistochemistry to characterise the amyloidogenic protein. This method has facilitated the identification of multiple new amyloidogenic proteins. A newer technique, independent of the need for Congo red staining, relies on detection of both the molecular weight and spatial distribution of biomolecules and the use of a novel peptide filter to classify amyloid proteins in a less time and sample consuming manner (60).

Imaging

In the context of suspected amyloidosis, echocardiography remains a first-line screening investigation for the presence of cardiac amyloid but is relatively non-specific. Cardiac magnetic resonance and bone scintigraphy tracer imaging are highly specific and have revolutionised the diagnostic approach to amyloidosis. CMR may also have a role in monitoring changes over time via the hallmark pattern of late gadolinium enhancement (61). Extracellular amyloid deposits lead to a marked increase in the myocardial extracellular volume, which provides a quantitative estimate of the myocardial amyloid burden. Extracellular volume, along with pre-contrast T1 mapping, correlates with established biomarkers of disease severity, such as NT-proBNP and Troponin T, and predicts mortality (62). Furthermore, myocardial amyloid regression can be accurately documented by a reduction in T1 and extra-cellular volume – a novel modality to track the progress of a patient following treatment (63). On CMR, T2 imaging is a marker of tissue oedema and can act as a potential myocardial "biomarker" of amyloid oligomer or light chain proteotoxicity (61). Improvement in cardiac amyloid can be seen via these modalities as pictured in **Figure 1.1**. Novel CMR methods are redefining our ability to track cardiac amyloid with clear prognostic value.

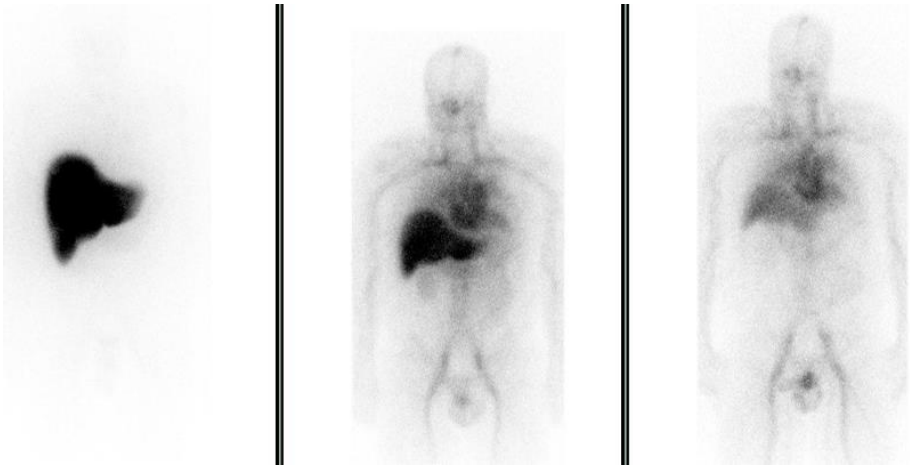
Figure 1.1: Cardiac magnetic resonance imaging modalities demonstrating improvement following a complete haematological response to chemotherapy. Image courtesy of Dr. Ana Martinez-Naharro and Professor Marianna Fontana



The use of radio-labelled bone-seeking tracers, such as [^{99m}Tc]-PYP or DPD, have transformed diagnosis of cardiac amyloidosis. These imaging techniques are highly sensitive for cardiac involvement in transthyretin amyloidosis and, in the absence of a monoclonal protein in serum or urine and normal serum FLCs, grade 2 or 3 myocardial radiotracer uptake is diagnostic of ATTR amyloidosis without the need for histological confirmation (64). On the contrary, these techniques lack sensitivity in AL amyloidosis with imaging positive in just 51% of patients with histologically confirmed cardiac AL amyloidosis (64). Of interest, ^{99m}-Tc-DPD uptake has also been reported in apolipoprotein A-I amyloidosis (65).

Imaging to quantify the amyloidogenic protein load is a valuable tool for both diagnosis and serial monitoring of changes in disease burden (seen in **Figure 1.2**). The UK NAC routinely uses ^{123}I-SAP scintigraphy to image visceral amyloid deposits within the liver, spleen, kidneys, adrenal glands and bones (66). This method is not useful to diagnose or track cardiac involvement.

Figure 1.2: Serial ^{123}I SAP scintigraphy in a patient who achieved a persistent complete haematological response to chemotherapy demonstrating regression of amyloid deposits within the liver over a 5-year period.



Whilst not yet routine practice, positron emission tomography-based imaging has emerged as a potential tool to evaluate the presence of amyloid deposits in both an upfront and post-therapy setting (67). Imaging using 18F-florbetapir (**Figure 1.3**) appears to be highly sensitive (67) but validation of this technique in larger studies is required. In the trial setting, a new radiotracer, designated p5+14, has been bound to ^{124}Iodine for use in PET-based imaging to demonstrate the presence of amyloid deposits. This tracer is a synthetic, basic polypeptide with 45 amino acids and forms an α-helix in the presence of highly sulfated glycosaminoglycans and amyloid fibrils, resulting in specific multivalent electrostatic interactions (**Figure 1.4**).

Figure 1.5 demonstrates a suggested algorithm for making a diagnosis of amyloidosis in suspected cases.

Figure 1.3: PET-CT imaging using 18F-Florbetapir demonstrating cardiac uptake in a patient with histologically-confirmed cardiac AL amyloidosis

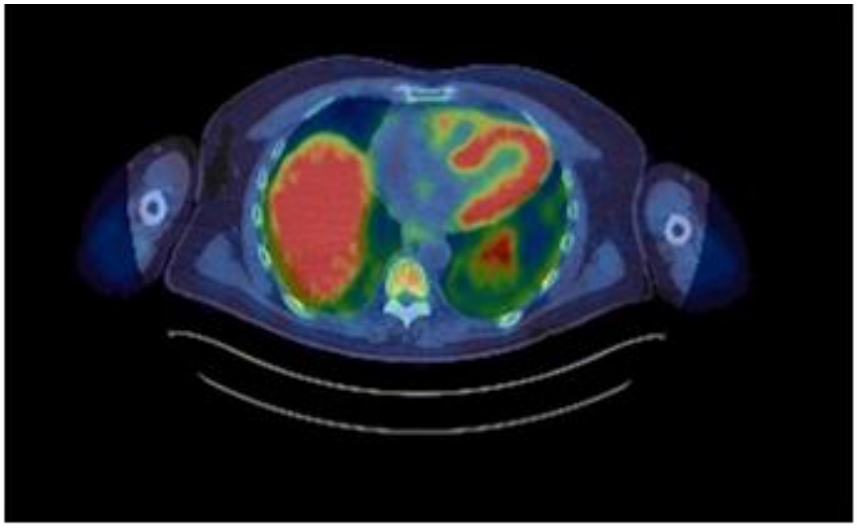


Figure 1.4: PET-CT imaging demonstrating uptake of p5+14 labelled with iodine-124 by amyloid in the liver. Image courtesy of Dr. Johnathan Wall.

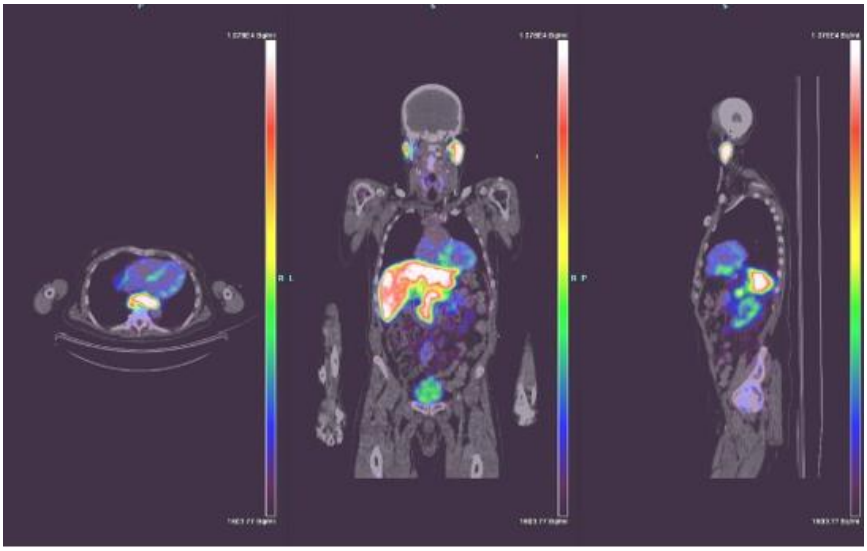
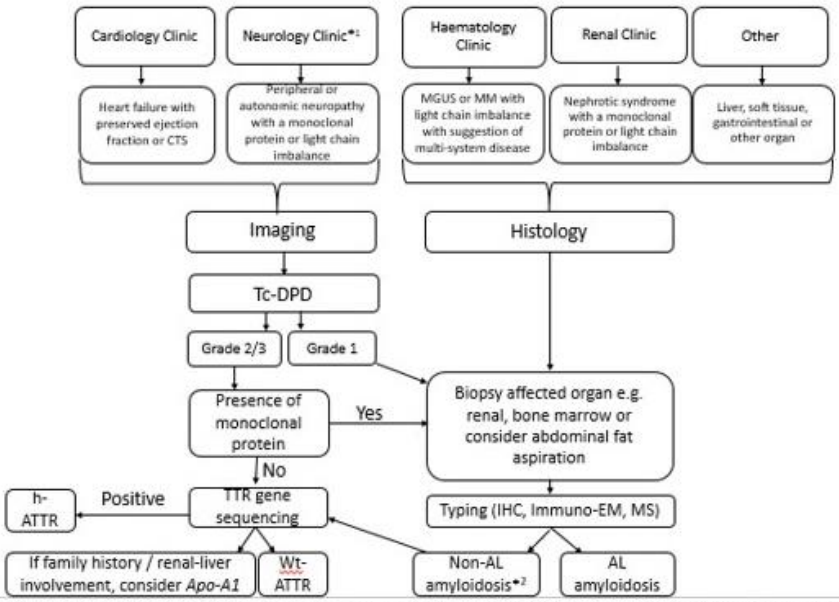


Figure 1.5: Diagnostic algorithm for diagnosis of amyloidosis.



*1: Consider DNA testing for Gelsolin-type amyloidosis in the presence of cranial neuropathies and/or corneal lattice dystrophy, especially in the context of family history of similar symptoms
*2: Consider DNA testing for fibrinogen-alpha chain amyloidosis in the presence of renal (+/-liver) involvement, especially in the presence of a family history of renal failure

Abbreviations: CTS, carpal tunnel syndrome; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; Tc-DPD, or ^{99m}-technetium-3,3-diphospono-1,2-propanodicarboxylic acid; h-ATTR, hereditary transthyretin amyloidosis; Wt-ATTR, Wild-type transthyretin amyloidosis; Apo A-1, Apolipoprotein A-1 amyloidosis; IHC, immunohistochemistry; Immuno-EM, immune-electron microscopy ;MS, mass spectrometry.

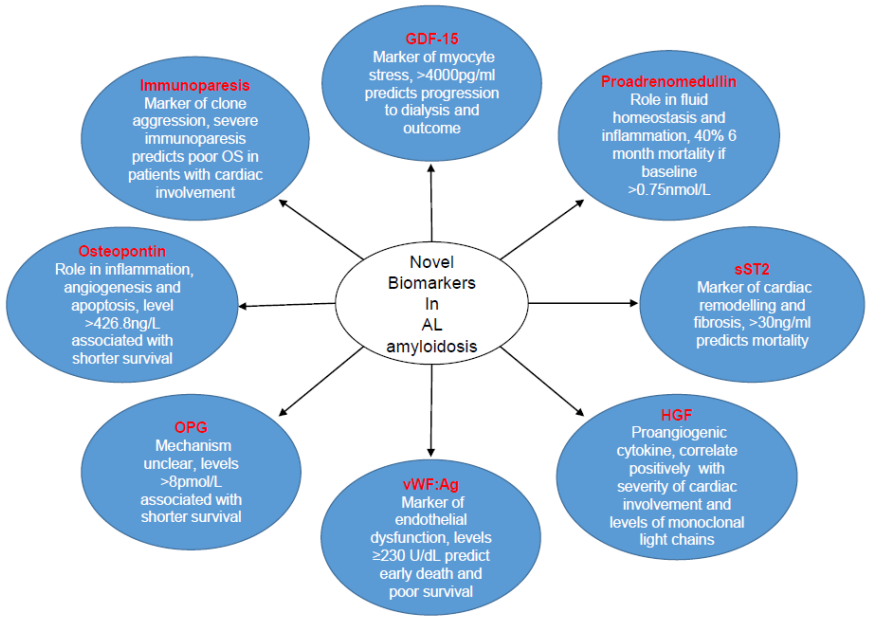
Risk stratification in AL amyloidosis

Biomarkers

Cardiac involvement is the major determinant of prognosis in AL amyloidosis and consequently, the cardiac biomarkers, NT-proBNP and Troponin, form the basis of the Mayo staging systems (20, 21). NT-proBNP has also been validated as a marker of cardiac response following treatment (68). However, it is exquisitely sensitive to a large number of factors that affect fluid balance, inclusive of renal dysfunction, making serial monitoring challenging. Lately, it has been demonstrated that depth of organ response in the heart, kidney and liver correlate with prolonged survival, which has led to the development of new proposed organ response criteria (69).

An increasing pool of biomarkers have been reported to have prognostic value in AL amyloidosis and include growth differentiation factor-15, proadrenomedulin, osteopontin, hepatocyte growth factor, soluble suppression of tumorgenicity 2, von Willebrand factor antigen, osteoprotegerin and immunoparesis (70-77) (**Figure 1.6**). These markers warrant further investigation in large case series' to provide a more accurate assessment of individual risk. At present, none of these novel markers have been incorporated into routine practice.

Figure 1.6: Novel Biomarkers in AL amyloidosis



Measurement of the underlying clone and clonal markers

The monoclonal protein free light chains are the drivers of disease in AL amyloidosis but it is the underlying biology of the clonal plasma cells that determines response to treatment, duration of response and outcomes at relapse.

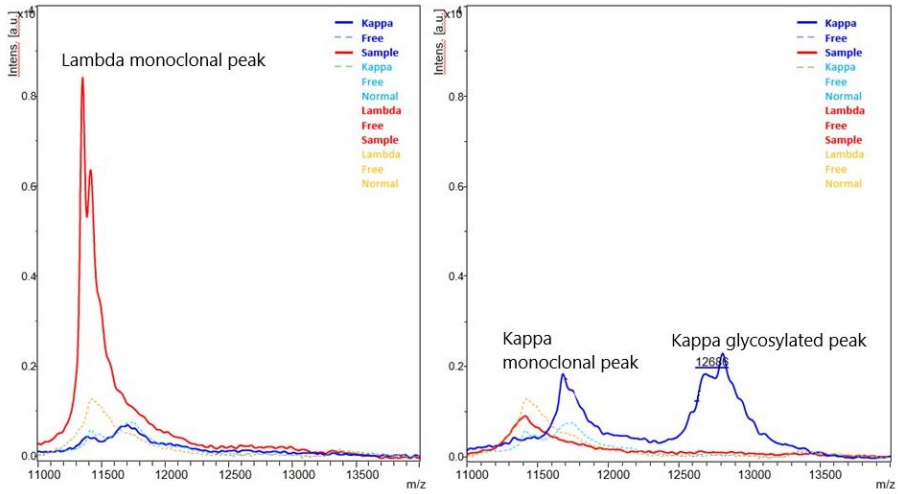
Advances in Light chain measurements

The development of assays to measure the total kappa and lambda free light chains (both monoclonal and normal polyclonal FLC) were transformative in the management of AL amyloidosis. Numerous assays are now available but two methods (Freelite™ by the Binding Site Group, Birmingham, UK and another immunoassay by Siemens Healthcare diagnostics, Germany) are most widely used. The assays use antibodies against hidden epitopes expressed on the light chain

molecule. There are persisting challenges with antigen excess leading to non-linearity and resultant under or over estimation of the monoclonal protein. There is a large coefficient of variance between centres (78). However, the critical failing of these assays is their inability to distinguish between the monoclonal and polyclonal components making up the total reported measurement of the FLC, which limits utility of the FLC measurement in patients with low level disease.

Mass spectrometry can be applied in peripheral blood to identify the monoclonal component of the involved FLC (**Figure 1.7**). The Mayo group pioneered a matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a simple and sensitive method to detect serum monoclonal proteins, called MASS-FIX. This is a highly automated, robust and reliable technique that can be easily adopted for high throughput testing. The method is far more sensitive than traditional electrophoresis and immunofixation (79). This modality has been developed further using FLC beads and a matrix-assisted laser desorption ionization time-of-flight mass spectrometry method to characterise monoclonal light chains, which is highly sensitive and can detect disease in patients whose clone is only detectable in bone marrow by flow cytometry-based MRD testing (80). In future, these methods may allow for more accurate monitoring of patients and formulation of early treatment decisions.

Figure 1.7: Mass Spectrometry demonstrating 1) A monoclonal lambda light chain peak 2) A monoclonal and glycosylated kappa light chain peak



Assessment of the plasma cell clone

Numerous studies have focused on the impact of clonal biology on patient management. In AL amyloidosis, the presence of myeloma-defining features such as increased bone marrow plasma cells (>10%) or the presence of co-existent hypercalcaemia, renal dysfunction, anaemia or lytic bone lesions define equally high-risk populations (81). Furthermore, the presence of circulating plasma cells by multi-parametric flow cytometry at diagnosis adversely impacts OS (although this is overcome by a good response to chemotherapy of VGPR or better) (82).

Patients with AL amyloidosis can be further risk-stratified based upon cytogenetic abnormalities. In the largest cohort reported to date (n=692), the translocation *t (11; 14)* was detected in 49% of patients whilst monosomy *13/del (13q)* was seen in 36% and trisomies in 26% by interphase fluorescence in situ hybridization (83). The presence of *t (11; 14)* is associated with a poorer response to bortezomib

and immunomodulatory drugs (83). A gain of *1q21* is also reported to be an adverse marker (84). The biological basis of these findings remains unclear and further research is needed to allow for the development of targeted therapies.

Whole exome sequencing has demonstrated 21 mutated genes in common between multiple myeloma and AL amyloidosis whilst also identifying 4 recurrent mutations in AL amyloidosis patients: *PCMTD1*, *C21orf33*, *NLRP12* and *NRAS* (85). Inferior overall survival has also been attributed to the presence of ASB15, ASCC3 and HIST1H1E (86). A large genome-wide association study in 1229 patients identified single nucleotide polymorphisms at 10 loci with *rs9344*, a promotor of *t (11; 14)*, the most significant (87). Whilst there appears to be no unique genomic signature of AL amyloidosis *per se*, further genetic sequencing studies are needed to increase understanding of the drivers behind AL amyloidosis, provide prognostic information and identify targets for future therapies.

Management of systemic AL amyloidosis

The management of patients with systemic AL amyloidosis requires a multi-disciplinary approach inclusive of haematologists, cardiologists, nephrologists and neurologists, as required. In addition to amyloid-directed chemotherapy, optimisation via supportive therapy is critical. Key elements may include careful fluid balance, blood pressure control and patient education.

Goals of therapy and response assessment

The aim of therapy is complete suppression of the underlying B-cell clone to reduce the production of amyloidogenic light chains and halt consequent tissue damage. This approach allows for a gradual organ response and longer overall survival. The avoidance of diagnostic delays and prompt implementation of therapy

remains crucial to minimise resultant organ damage. Furthermore, rapid haematological responses are associated with improved outcomes in patients with advanced disease (88). The advantages of a particular cytotoxic treatment must be balanced against its toxicity, particularly in the context of compromised baseline organ function, which may be significant secondary to amyloid deposition.

Standardised assessment of disease status is key to inform treatment intensity and choice, both at baseline and in the relapse setting. In recent years, it has become increasingly apparent that deeper haematological responses improve outcomes. Reduction of the involved free light chain to <20mg/L or dFLC to <10mg/L translates to superior overall survival and organ responses, over and above a traditional CR by consensus criteria (89, 90). A minority of patients present with a dFLC ≤50mg/L, which poses a challenge in terms of disease tracking.

Minimal residual disease assessment provides a more sensitive method of assessing disease status via flow cytometry or NGS. The persistence of ≥0.1% circulating monoclonal plasma cells following chemotherapy is a negative predictor of both progression-free and overall survival (91). Furthermore, the detection of bone marrow plasma cells by flow cytometry negatively impacts PFS in patients achieving a CR (92). Impaired organ recovery is seen in patients who are MRD positive by flow cytometry (93). The role of both flow cytometry and NGS remains unclear and require further study before incorporating their use into routine treatment decisions.

Plasma Cell Directed Therapy

Autologous Stem Cell Transplantation

Autologous stem cell transplantation is the standard of care for eligible patients with systemic AL amyloidosis. Treatment-related mortality has decreased over time with improved patient selection (important exclusion criteria are listed in **Table 1.2**). A United States registry study reports a transplant-related mortality of 5% from 2007-2012, down from 20% in the period 1995-2000 (94). Whilst ASCT leads to durable remissions in patients achieving a deep haematological response (CR or VGPR) (OS 7.6 years in a US series (95) and 11.6 years in UK series (96)), a CR is seen in just a third of patients (34.8% in the Boston series (95)).

Both induction chemotherapy and post-transplant consolidation have been implemented to overcome limitations of ASCT. The administration of upfront bortezomib-based induction chemotherapy leads to CR rates of 63% with median PFS and OS not reached at 36 months (97). Initial treatment with chemotherapeutic agents can render patients who were initially deemed transplant-ineligible due to organ dysfunction to proceed to ASCT with a PFS of 54 months (96). Autologous stem cell transplantation may still be suitable for patients refractory to upfront bortezomib (PR or worse), with one small study reporting a 42% CR rate in 12 patients (98). Conversely, bortezomib consolidation therapy in patients in a VGPR or worse following ASCT alone, led to one-third upgrading to a CR subsequently (99) The optimal timing, nature and duration of additional therapy around ASCT remains unclear and presents an ongoing dilemma when managing this patient group.

Table 1.2: Exclusion Criteria for ASCT

	UK NAC (100)	Mayo (101)	Boston (95)	Heidelberg (100)
Cardiac	NT-proBNP >1000ng/L	Troponin T >0.06ng/ml	Ejection fraction <40% Uncompensated heart failure / resistant arrhythmia	Severe cardiac failure
Renal	Serum albumin <20g/L eGFR <40ml/min	CrCl <30ml/min		
Blood Pressure	Systolic ≤90mmHg	Systolic <90mmHg	Systolic <90mmHg	Systolic ≤90mmHg
Performance Status	ECOG PS >2	ECOG PS >2 NYHA III-IV	ECOG PS >2 unless limited by peripheral neuropathy	ECOG PS ≥2
Other	Large load on SAP scintigraphy	>2 major organs involved Age >70years	Symptomatic pleural effusions Oxygen saturations <95% air or lung diffusion capacity <50% predicted	Gastrointestinal bleeding

Standard chemotherapeutic approaches

Bortezomib-based combination therapy is established as the first-line treatment for the majority of patients with systemic AL amyloidosis. **Table 1.3** shows the treatment combinations available. In a randomised phase III trial, newly diagnosed patients treated with bortezomib-melphalan-dexamethasone had CR/VGPR rates of 53% compared to 28% with melphalan-dexamethasone alone (102). The triplet of bortezomib-cyclophosphamide-dexamethasone demonstrated haematological, renal and cardiac response rates of 60%, 25% and 17% respectively in a large European collaborative study (103). Furthermore, the UK NAC has reported haematological, renal and cardiac response rates of 65%, 15.4% and 32.5% in a very large series of 915 patients (104). Bortezomib is the key drug here with the Greek amyloid group finding that the addition of cyclophosphamide to this combination does not significantly improve efficacy or survival (105). A rapid response to bortezomib-based therapy can improve outcomes even in advanced cardiac patients (median OS improving from 5m to 26m in patients achieving a CR/VGPR by end of one month (88)).

Immunomodulatory drugs are routinely used in the relapse setting. The efficacy of lenalidomide-dexamethasone was first reported in 2006 (106, 107). Using this combination, the Greek amyloid group demonstrated 51% haematological, 22% renal, 7% liver and 3% cardiac response rates (108). In combination with melphalan and dexamethasone, haematological response rate was similar (58%) but only 8% achieved an organ response. This combination was highly toxic with 40% of patients dying within months of therapy due to acute cardiac events and a median OS of 1.75 months for Mayo stage III patients (109). However, a further study of lenalidomide-melphalan-dexamethasone in untreated transplant ineligible patients yielded superior outcomes with a 68% haematological response and 48% organ response. In this

group, median OS was 67.5 months. There was just one cardiac death after 3 cycles of chemotherapy despite 18 patients (36%) having Mayo stage III disease (110). However, stage III patients still had a PFS of <12 months and the proportion of patients with stage IIIb disease was not specified. In both studies, the lenalidomide dosing (10mg) and frequency was the same but the German group used a lower melphalan dose of 0.15mg/kg as opposed to 0.18mg/kg, which may have impacted upon the level of toxicity reported.

Pomalidomide is rapidly acting and has shown a survival advantage in heavily pre-treated patients (111-113). Treatment with pomalidomide-dexamethasone yields a 66% haematological response rate with a median PFS of 15 months although no patients achieved a CR with pomalidomide alone (114). Further evaluation of pomalidomide as part of combination chemotherapy is required to assess its efficacy in this setting although toxicity may be an issue in heavily pre-treated patients with a UK NAC study reporting a 41.1% (7/19 evaluable patients) discontinuation rate due to adverse events in patients with a median of 4 prior lines of therapy.

The addition of clarithromycin to IMiD-based therapies has demonstrated efficacy. One study examined patients with either multiple myeloma (n=32) or AL amyloidosis (n=17) demonstrating a 94% haematological and 47% organ response rate in patients with AL amyloidosis (35% haematological response prior to the addition of clarithromycin in the same cohort) (115). However, the recent report of increased mortality when clarithromycin was added to lenalidomide-dexamethasone in multiple myeloma(116) suggests a need for caution when taking this approach.

Table 1.3: Treatment regimens for patients with AL amyloidosis.

	Study	Chemotherapy	Prior lines of therapy	Patient No.	Haematological response (CR)	Organ Response	Median PFS	Median OS
Bortezomib	**Kastritis et al 2017 (105)**	Cyclophosphamide-bortezomib-dexamethasone	0	42	78% (21%)	Cardiac: 21%, Renal 41%		36m
		Bortezomib-dexamethasone	0	59	68% (27%)	Cardiac 29% , Renal 43%		33m
	Palladini et al 2015 (103)	Cyclophosphamide-bortezomib-dexamethasone	0	230	60% (23%)	Cardiac 17% , Renal 25%		55% (5 yr)
	Manwani et al 2019(104)	Bortezomib-based therapy (95% CyBorD)	0	915	65% (25%)	Cardiac 32.5%, Renal 15.4%	22m	72m
	Palladini et al 2014 (117)	Bortezomib-melphalan-dexamethasone	0	87	69% (42%)	Cardiac 16% , Renal 16%	TNT / death: 39 m	NR
Carfilzomib	**Cohen et al 2016 (118)**	Carfilzomib	≥1	28	63% (12%)	Renal 12%		
Ixazomib	**Sanchorawala et al 2017 (119)**	Ixazomib-dexamethasone	3 (1-8)	27	52% (9.5%)	Cardiac 45% Renal 45%	14.8m	85% (1 yr)
	Dispenzieri et al, 2022 (120)	Ixazomib-dexamethasone	1-2	168	53% (26%)	Cardiac 18% Renal 24%	11.2m	NR
Thalidomide	**Wechalekar et al 2007(121)**	Cyclophosphamide-thalidomide-dexamethasone	0-1	75	74% (21%)	Cardiac 0%, Renal 23%	21m	41m
Lenalidomide	**Sanchorawala et al 2006 (107)**	Lenalidomide+/-dexamethasone	1 (0-5)	34	67% (29%)	Cardiac 7.7%, Renal 41%,		
	Dispenzieri et al, 2007	Lenalidomide+/-dexamethasone	1 (0-3)	23	40.9%	Cardiac 14.3%, Renal 25%		
	Mahmood et al 2014 (122)	Lenalidomide-dexamethasone	2 (1-6)	84	61% (20%)	Cardiac 12% , Renal 55%	73% (2 yr)	84% (2 yr)
	Kastritis et al 2018 (108)	Lenalidomide-dexamethasone	1 (1-4)	55	51% (5.5%)	Cardiac 3%, Renal 22%		25m
	Basset et al, 2021(123)	Lenalidomide-Dexamethasone	2 (1-6)	260	31% (4%)	Cardiac 0% Renal: 23%	9m	32m
	Kumar et al 2012 (21)	Cyclophosphamide-lenalidomide-dexamethasone	0 (0-2)	35	60% (11%)	23% Cardiac, 31% Renal	28.3m	37.8m
	Hegenbart et al 2017 (110)	Lenalidomide-melphalan-dexamethasone	0	50	68% (18%)	48%	25.1m	67.5m
Pomalidomide	**Dispenzieri et al 2012 (111)**	Pomalidomide-dexamethasone	2 (1-8)	33	48% (3%)	15% Cardiac, 17% Renal	14m	28m
	Sanchorawala et al 2016 (112)	Pomalidomide-dexamethasone	2 (1-6)	27	50% (33%)	Renal 7.1%	17.8m	NR
	Palladini et al 2017 (113)	Pomalidomide-dexamethasone	2 (1-7)	28	68% (4%)	Renal 17%	16m	26m
	Sharpley et al 2018 (114)	Pomalidomide-dexamethasone	4 (1-7)	29	66% (0%)	Cardiac 38% , Renal 44%	15m	27m
Daratumumab	**Kaufman et al 2017 (124)**	Daratumumab	3 (1-5)	25	76% (36%)			
	Abeykoon et al 2019 (125)	Daratumumab monotherapy	3 (1-8)	22	78% (14%)	Cardiac 43%, Renal 18%	NR	NR

		Daratumumab combination		22	88% (19%)	Cardiac 46% , Renal 36%	NR	NR

Novel chemotherapeutic agents

Proteasome Inhibitors

Newer proteasome inhibitors, Carfilzomib and Ixazomib, have limited evidence for use in AL amyloidosis. Carfilzomib is less associated with neurotoxicity than bortezomib. Haematological and organ responses in 63% and 21% patients respectively have been reported in a multicentre phase I/II study. However, toxicity was significant with 71% patients experiencing grade 3/4 toxicity, which was most commonly cardiac or pulmonary (118). Subsequently, improved tolerability and response rates have been reported in a phase I study of weekly carfilzomib in combination with thalidomide and dexamethasone (126). Further combinations of weekly carfilzomib with newer IMiDs or daratumumab require further study.

Ixazomib is an oral proteasome inhibitor with efficacy in the relapsed or refractory setting. Sanchorawala *et al* (2017) reported a 52% haematological response and 56% organ response (50% renal, 50% cardiac) with a median PFS of 14.8 months (119). However, a subsequent phase III clinical trial of ixazomib-dexamethasone compared to a regimen of physicians choice in relapsed AL amyloidosis did not meet the primary end point in a planned intern analysis. However, the results demonstrate improved PFS (11.2 v 7.4 months, p=0.043), TNT (26.5 v 12.5 months, p=0.027) and prolonged time to vital organ deterioration (34.8 v 26.1 months, p=0.012) with Ixazomib-Dexamethasone (127). These newer proteasome inhibitors have promising advantages in patients with neurotoxicity and those who would benefit from an oral agent to minimise visits to their haematology centre but further work is required to fully characterise their efficacy and toxicity in larger groups of patients.

Anti-CD38 monoclonal antibodies

Daratumumab, an anti-CD38 monoclonal antibody, is showing remarkable promise in AL amyloidosis. One study demonstrated a 76% haematological response rate (36% CR) with a median response time of 1 month in heavily pre-treated patients inclusive of 72% with cardiac involvement (124). The Mayo clinic have reported impressive haematological response rates of 78% with daratumumab monotherapy and 88% with combination therapy (addition of bortezomib, lenalidomide or pomalidomide)(125). Whilst best cardiac response rates were similar in both groups, they occurred earlier (8.3 v 14.6 months) in the monotherapy group. The treatment was well tolerated although 22% of patients experienced significant infusion reactions.

The ANDROMEDA trial examined frontline subcutaneous daratumumab in combination with CyBorD. In 388 patients, there was a significantly higher response rate in the daratumumab arm (53.3% vs. 18.1%) vs. the control group, who received CyBorD alone. This study also demonstrated higher rates of survival from major organ deterioration or haematological progression in the intervention arm (128).

Venetoclax

The anti-apoptotic protein, BCL-2, is expressed at higher levels in patients with plasma cell dyscrasias associated with the *t (11; 14)* translocation. Approximately half of patients with AL amyloidosis harbour this mutation. Venetoclax is a BCL-2 inhibitor thus providing the rationale for its use in this setting. Of 7 patients treated with venetoclax for AL amyloidosis (alone or in combinations including bortezomib and lenalidomide), 5 achieved a haematological response (2 CR, 3 VGPR). However, 2 patients discontinued therapy (1 cytopenia, 1 suboptimal

response) and 4 patients suffered gastrointestinal side effects (129). A further report of 2 heavily pre-treated patients, receiving venetoclax in combination with a proteasome inhibitor, documented a CR in both patients. One patient stopped treatment due to pneumonia after cycle 2 whilst a second stopped treatment due to the discontinuation of the BELLINI trial. The former has remained in CR, without further treatment, almost a year later (130). Finally, a third series of venetoclax +/- bortezomib in relapsed-refractory cardiac AL amyloidosis presented evaluable outcomes in 4/7 patients (2 received 1 cycle only, 1 died of pneumonia after cycle 1) with a 50% response rate sustained at 76 and 713 days (131). These early results are promising but a degree of caution is required given early toxicity data.

IgM associated AL amyloidosis

Approximately 5-7% of cases of systemic AL amyloidosis are associated with an underlying immunoglobulin M monoclonal protein, which usually occurs secondary to a lymphoplasmacytic lymphoma. In these patients, cardiac involvement occurs less commonly (45%) whereas neuropathic (28%) and lymph node (20%) involvement are relatively more common. Whilst IgM-associated AL amyloidosis is a separate entity, the goal of reducing the free light chains and monoclonal protein remains prognostic. Large collaborative studies to guide therapy in this subgroup of patients are lacking but the combination of rituximab with bendamustine, bortezomib, cyclophosphamide and purine analogues are increasingly favoured (132). Whilst responses to both alkylators and purine analogues are poor in this setting, the overall response rate with rituximab-bendamustine (133) and rituximab-bortezomib (134) have been reported to be 76% and 78% respectively on single centre retrospective analyses. Further study is needed to determine the optimal combination therapy in this difficult-to-treat subgroup of AL amyloidosis.

Amyloid fibril-directed therapy and the challenge of trial endpoints

AL Amyloidosis

NEOD001, a drug that binds amyloidogenic light chains and promotes phagocytic clearance in vitro (135), failed to show efficacy in prospective trials and development has been discontinued . The PRONTO study used cardiac best response, assessed by NT-proBNP, as a primary endpoint despite the high level of variability associated with this biomarker. Furthermore, NT-proBNP increases after chemotherapy in 71% of patients at six months (136) thus early measurements can lead to false positive results. Whilst NT-proBNP predicts clinical outcome (68), these factors highlight the challenges associated with its use as a study endpoint. Analysis of the Phase 3 VITAL study of NEOD001, in addition to standard care, suggests a survival benefit in high-risk Mayo Stage IV patients thus additional clinical studies of NEOD001 may be warranted in future (137).

A phase 1 trial of CPHPC (miridesap), with dezamizumab, a humanized monoclonal anti-SAP antibody, demonstrated hepatic and renal clearance of amyloid deposits with reduction of the splenic amyloid load and improved hepatic function (138). However, the trial assessing this combination (NCT03044353) in cardiac amyloidosis was stopped after an interim data review cited an unfavorable risk-benefit. The chimeric fibril-reactive monoclonal antibody, CAEL-101 (formally 11-1F4), has also been shown to be safe in a phase 1 setting with interim analysis reporting reduction in the amyloid burden with rapid improvement in organ function (139). A study to evaluate the efficacy and safety of CAEL-101 in patients with Mayo IIIa AL Amyloidosis is open to recruitment (140).

Further work aiming to better evaluate the structure and pathogenesis of light chain protein misfolding is also underway. Two studies have used cryo-electron microscopy mapping of tissue-extracted amyloid fibrils from patients with AL amyloidosis to provide greater insight into the mechanism of protein misfolding. This work may lead to the development of novel ligands providing a foundation for future amyloid fibril-directed therapy (141, 142).

Doxycycline interferes with amyloid fibril formation and has been shown to reduce early cardiac mortality in AL amyloidosis without impacting haematological response (143). A further trial evaluating the addition of doxycycline to bortezomib-based therapy (NCT03474458) is underway.

RNA inhibitors and protein stabilisers in ATTR and AL amyloidosis

Two strategies have transformed the therapeutic scenario in ATTR amyloidosis. Transthyretin stabilisers have been used to slow disease progression with some success. Diflunisal, a non-steroidal anti-inflammatory medication, and tafamidis, a thyroxine-like transthyretin-stabiliser, reduce neurological progression and improve quality of life scores (144, 145). Tafamidis is licenced for this indication in Europe. A phase III study demonstrated a significant survival benefit for patients with cardiac ATTR treated with tafamidis, which has led to tafamidis being the first licensed treatment for this indication. Exciting gene-silencing therapies (patisaran (13)and inotersen (14)) selectively switch off transthyretin production and are now licenced for patients with neuropathic hATTR amyloidosis. Both agents have demonstrated highly significant improvements in neurological and quality of life scores. Patisiran also decreased mean left ventricular wall thickness, global longitudinal strain, NT-proBNP and adverse cardiac outcomes (146) suggesting an

effect on patients with ATTR and associated cardiac involvement. Longer acting gene silencers (vutrisiran) and more potent transthyretin stabilisers (AG-10) are in clinical trials.

AL amyloidosis has trailed ATTR in these crucial therapeutic aspects. Recently, high-throughput screening and characterisation identified several small molecules that kinetically stabilise FLCs by binding at the V-domain–V-domain interface in both kappa and lambda light chains providing the first step to a potential FLC stabilising approach (147). Whilst pre-clinical work suggests there is potential for RNA inhibitors in reducing FLC production (148), this remains challenging to translate into *in vivo* models.

Organ transplantation

Organ transplantation was historically controversial in systemic amyloidosis given its potential to recur within the transplanted organ. More recently, promising outcomes have been reported across multiple amyloid subtypes in the context of end-stage organ dysfunction. In patients with AL amyloidosis, organ transplantation can be considered in patients achieving a pre-transplantation complete or very good partial haematological response to therapy in order to maximise the suppression of the amyloidogenic light chain and maximise the time to recurrent amyloid-related organ dysfunction in the graft (149).

In the setting of renal transplantation, a median graft survival in AA, AL, apolipoprotein A-I and fibrinogen amyloidosis of 10.3, 5.8, 13.1 and 7.3 years respectively has been reported (54) although patient numbers outside of AA amyloidosis are small. Furthermore, this paper evaluated patients who received a transplant between 1978 and 2011 since which time, particularly in the setting of AL amyloidosis, treatments have vastly improved and a wider range of novel agents are available. More recently, a US series published outcomes of 49 patients transplanted 1987-2017 showing an improved graft survival of 10.4 years in patients achieving a CR/VGPR (30/49 [61.2%] patients) yet large case series (150) particularly with a focus on outcomes of patients transplanted exclusively in more recent years, are lacking.

Orthotopic liver transplantation has historically been implemented for ATTR amyloidosis as a disease-modifying therapy given that the transthyretin protein, which forms the basis of the amyloid fibrils in question, is formed within the liver. A large review of over 2063 procedures over 23 years, reported a 5-year survival of 100% in V30M patients and 59% in non-V30M patients with ATTR amyloidosis.

Whilst longer disease duration and co-existent cardiomyopathy impacted negatively on outcome, successful liver transplantation usually halts disease progression (12). In AL amyloidosis, published evidence is more limited and transplantation does not address the underlying aetiology or prevent extra-hepatic organ dysfunction. One study reported, a decade ago, outcomes of 9 patients who underwent liver transplantation for hepatic amyloid with a 1 and 5-year survival of 33% and 22% respectively (151).In apolipoprotein A-I amyloidosis, there are a limited series' of cases of hepato-renal transplantation. A UK series reported outcomes of 2 patients with combined transplants alive at 7.8 and 3.8 years post-transplantation with functioning grafts in both cases (152).

Cardiac transplantation is also reported across amyloid subtypes. In ATTR amyloidosis, cardiac transplantation has historically been accompanied by a liver transplant. A series of 52 patients, taken from the Familial Amyloid Polyneuropathy World Transplant Registry, receiving both heart and liver transplants had a 62% overall survival at 4.5 years (153). A more recent Italian study reported 5-year survival of 82% in 14 patients (154). In AL amyloidosis, a UK-based study of 17 AL amyloid patients reported 5-year survival of 20% in patients not receiving additional chemotherapy and 36% in those who did (155). Finally, in apolipoprotein A-I amyloidosis, there are limited case reports reporting successful combination transplants inclusive of 2 within the UK series reporting functioning grafts at 4.5 and 13.0 years post-transplantation (152).

In summary, recent advances in the diagnosis and treatment of amyloidosis, hold promise. Failure of early detection remains a critical barrier to improving outcomes. The adoption of amyloid-specific imaging has led to a marked increase in the detection of wtATTR amyloidosis. New methods to detect monoclonal proteins assist both diagnosis and monitoring during treatment. In AL amyloidosis, response assessment and tracking of organ damage is improving whilst new MRD-based methods assist in the detection of early relapse and may guide the initiation of next line therapy prior to the deposition of significant further amyloidogenic protein and associated organ dysfunction.

Rapid reduction in amyloidogenic light chains to preserve organ function in AL amyloidosis is critical. Risk stratification to direct therapy has improved outcomes in high-risk AL patients. Both novel agents and new combinations of therapies show promise in achieving rapid responses and improving survival with a number of clinical trials underway investigating these agents. There have been significant therapeutic advanced in ATTR treatment, which may change the disease trajectory. Organ toxicity limits life expectancy in both AL and ATTR amyloidosis. The development of treatments that directly remove amyloidogenic protein from the circulation or accelerate clearance of tissue amyloid deposits still remains a horizon to be reached.

Aims and scope of thesis

Outcomes are improving for patients with systemic amyloidosis. Advances in diagnostic techniques, stringent supportive care and novel disease-modifying agents may all be contributory. The introduction to this thesis provides an overview of the main subtypes, investigation and treatment pathways and latest advancements in the field of amyloidosis. The remainder of the thesis focuses upon three key areas; namely, the utility of diagnostic screening biopsy and prognostic markers in amyloidosis, the use of novel therapies in amyloidosis and finally, organ transplantation.

Systemic amyloidosis is a rare disease and diagnostic delays remain an issue of critical importance in the drive to improve patient experience and outcomes. The use of target organ biopsies to prove the diagnosis may contribute towards delays and represents a higher-risk option than screening biopsies (156), which may be performed at the bedside. Chapter 3 focuses on the diagnostic sensitivity of screening biopsy in both AL and ATTR amyloidosis. The use of abdominal fat, bone marrow trephine and gastrointestinal biopsies are analysed in the context of amyloid subtype, organ involvement and disease burden.

The following chapters (4 and 5) concentrate on the prognostic utility of functional markers in systemic AL amyloidosis both at baseline and following amyloid-directed therapy. This section will also focus on the use of such markers to identify groups with a particularly poor prognosis at baseline with a view to informing treatment decisions. Cardiac involvement by amyloidosis remains the major determinant of prognosis (23) and yet organ response is predominantly assessed via an improvement in NT-proBNP, a biomarker that is incredibly sensitive to changes in

fluid balance. Longitudinal strain, a marker of longitudinal cardiac function, may be a more appropriate functional biomarker to assess organ response in patients with cardiac AL amyloidosis. Chapter 4 concentrates on evaluating the utility of this functional marker in AL amyloidosis to determine prognosis at both baseline and follow up. This chapter also looks at the impact of haematological response on change in longitudinal strain and whether strain can provide incremental prognostic information above that afforded by a traditional biomarker-based organ response. Chapter 5 goes on to examine the prognostic impact of functional capacity as assessed by the 6-minute walk test. This simple assessment of patient function could provide an additional objective outcome measure that is lacking at present. In Chapter 5, the value of walk testing is assessed in the context of both cardiac and extra-cardiac AL amyloidosis. The impact of the 6MWT is evaluated in the context of organ involvement, haematological response and traditional organ response criteria.

Patients with organ impairment secondary to amyloidosis, often have a significant disease burden, which impacts function. In addition to an objective functional assessment (the 6MWT), a standardised assessment of the physical and mental impact of the disease on health-related quality of life may be of great value in better understanding patient experience. Furthermore, such an assessment may also contribute prognostic information at baseline and following chemotherapy. Chapter 6 provides as assessment of health-related quality of life, as established by the SF-36 v2, at baseline, during chemotherapy and following cessation of chemotherapy. This chapter seeks to improve the understanding of the prognostic impact of quality of life evaluation and its relationship with organ involvement and both haematological and organ response.

Section 2 focuses on the outcomes of patients with systemic AL amyloidosis receiving novel agents in the relapsed/refractory setting to treat their disease. The need for a wider range of such agents is increasing as patients with AL amyloidosis survive longer (23) and require further therapy at relapse. Chapter 7 evaluates the use of the novel triplet, ixazomib-lenalidomide-dexamethasone, the use of which is previously unpublished in patients with systemic AL amyloidosis. Chapter 8 examines the use of daratumumab monotherapy in a similar cohort of patients with relapsed/refractory disease. The impact of these novel treatment regimens on survival is reported.

Finally, section 3 focuses on the use of organ transplantation in amyloidosis. Organ transplantation was historically controversial due to the potential of the amyloid to recur in the graft. However, improved patient selection and novel agents with the potential to achieve deeper haematological responses have improved outcomes. In AL amyloidosis, one-third of patients with renal involvement progress to end-stage renal failure (157) and yet published outcomes of renal transplantation remain sparse. In Chapter 9, the outcomes of a large cohort of patients with systemic AL amyloidosis who received a renal transplant within the last 15 years are reported. Chapter 10 reports the natural history and use of organ transplantation in Apolipoprotein A-I amyloidosis in a large cohort of 57 patients inclusive of 18 patients who received an organ transplant. This is the largest series of transplanted patients with this amyloid subtype studied to date.

Chapter Two: Materials and Methods

Declaration

I have designed the studies, collected data and performed statistical analysis within my role as a clinical research fellow at the UK National Amyloidosis Centre, University College London Medical School (Royal Free Campus).

Several diagnostic methods used to collect data for use in these thesis were conducted by others, specifically:

1. Histological and immunohistochemical analysis was conducted by Janet Gilbertson and Nicola Botcher.
2. Genetic sequencing was performed by Dorota Rowczenio and Hadija Trojer
3. Echocardiography was performed by Brook Douglas, Babita Pawarova, Andreia Ismael and and Sevda Ward.
4. ^{123}I-SAP and ^{99m}TcDPD scintigraphy was performed by David Hutt and Florentina Grigore.
5. The 6-minute walk test was officiated by a number of trained health care assistants working within the NAC.
6. Quality of life questionnaires were administered to patients by Darren Foard.
7. Measurement of haematological and biochemical markers was performed by the laboratory service within the Royal Free Hospital.

Patients

All of the patients recruited for studies reported within in this thesis were seen at the UK National Amyloidosis Centre, London. Medical records were retrieved from a secure electronic database and anonymised for the purpose of these studies. Informed consent in the form of a written form was gained for all patients. Data

relating to death was updated on the electronic database based upon information from the Office of National Statistics.

Histology

Acquisition of tissue for diagnosis

Abdominal fat aspiration was performed as a screening biopsy in patients presenting with suspected amyloidosis without histological evidence of amyloidosis detected elsewhere or, in the case of suspected ATTR amyloidosis, not meeting non-biopsy criteria for diagnosis. Conversely, if histological material was available from a previous biopsy performed locally, these were received by the laboratory at the National Amyloidosis Centre as formalin-fixed paraffin blocks.

When abdominal fat aspiration was required, patients expressed verbal consent after which, the procedure was performed under aseptic technique using up to 5 millilitres of Lidocaine 1% to anaesthetise the skin. The fat aspirate was performed using a 16 gauge Microlance™ needle as previously described (158). Fat smears subsequently underwent Congo red staining and were formalin-fixed and double-embedded in agar before a paraffin block was produced. The process to diagnose amyloid thereafter is described below.

Congo red staining

Puchtler's method (159) was implemented to detect the presence of amyloid fibrils. Specifically, formalin-fixed de-paraffinised tissue, in sections 6µg in length, were counter-stained with haematoxylin under running water, following rehydration. The slides were then stained with Congo red solution after being placed in ethyl alcohol. The sections were dehydrated using increasing ethanol concentrations to

xylene before being mounted onto slides with DPX mounting media. Once dry, the stained slides were observed in a bright field under cross-polarised light. A known positive section, verified by laser micro-dissection and mass spectrometry was used as a positive control. Two experienced independent observers viewed the sections in order to confirm the presence of amyloid.

Immunohistochemistry and Mass spectrometry

Once amyloid deposits were confirmed, immunohistochemistry was used to determine amyloid subtype. In preparation, the amyloid-containing sections of 2µm fixed de-paraffinised tissue were cleansed, washed, incubated in aqueous hydrogen peroxide and finally, rinsed in a phosphate buffered solution containing 0.05% Tween (Calbiochem). A further incubation step, in normal non-immune serum from the relevant species from which the antibody was extracted (Vector Part of the ImmPRESS™ kit), was employed to eliminate non-specific tissue binding. After an overnight incubation with primary anti-sera at four degrees Celsius, sections were washed in phosphate buffered solution containing 0.05% Tween (Calbiochem) and labelled with secondary antibodies. A metal-enhanced DAB (Fisher Scientific solution) was used to visualise the resultant antibody-enzyme complexes. Immunohistochemical staining was done using a panel of monospecific antibodies against known amyloid fibril proteins inclusive of kappa and lambda light chains, transthyretin and apolipoprotein A-I. A positive control was always included. Congo Red overlay was used in duplicate sections whilst stained sections were counterstained in haematoxylin, ‘blued’ under running water and stained with Congo Red (160). Two experienced independent observers assessed the slides to determine positivity.

In a minority of cases whereby subtype could not be established by immunohistochemistry, laser capture micro-dissection and mass spectrometry, as previously described (161) was employed in order to confirm diagnosis as per previously published guidance.

Functional Assessments

Performance status was evaluated using the Eastern Co-operative Group guidance (162) (**Table 2.1**) whilst the New York Heart Association classification of Heart Failure (163) was used to classify symptoms in appropriate patients (**Table 2.2**). The 6-minute walk test was also employed for all eligible patients as per American Thoracic Society guidelines (164). Patients were asked to rest in a chair for 10 minutes prior to the commencement of the test. Patients were instructed to walk the length of a corridor and back repeatedly on a flat surface and at their own pace for a period of 6 minutes. Language used to provide instructions and to prompt the patient each minute was standardised as per guidance. Patients were excluded from participating in the 6MWT if they met criteria for an absolute or relative contraindication, namely unstable angina or myocardial infarct within the previous month, resting heart rate >120 beats per minute, systolic blood pressure >180mmHg and/or diastolic blood pressure >100mmHg. A lying and standing blood pressure and electrocardiogram was performed in all patients prior to commencement of the 6MWT to ensure participants were safe to proceed. The absolute distance walked in 6 minutes was recorded in metres, which was then used to derive a percentage of the predicted value for age, sex, height and weight (165).

Table 2.1: Eastern Co-operative Oncology Group Performance status (ECOG) (162)

Grade	ECOG Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about >50% of waking hours
3	Capable of only limited self-care; confined to bed or chair >50% of waking hours
4	Completely disabled; cannot carry out any self-care; totally confined to bed or chair
5	Dead

Table 2.2: New York Heart Association (NYHA) Functional Classification of heart failure (163)

Class	Patient Symptoms
1	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitations and/or dyspnoea
2	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity leads to fatigue, palpitations and/or dyspnoea
3	Marked limitations of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation and/or dyspnoea.
4	Unable to participate in any physical activity without discomfort. Symptoms of heart failure at rest. Discomfort increases with any physical activity.

Haematological Assessment

Assessment of clonal disease

All patients were assessed for the presence of a monoclonal protein in serum and urine and had sFLCs measured at baseline and each subsequent visit to the NAC. Additionally, patients were asked to send postal blood samples to the NAC on a monthly basis to allow for more regular clonal disease assessment. Serum protein and immunofixation electrophoresis were performed by the laboratory at the Royal Free Hospital via standardised procedures. Assessment of serum free light chains was performed using latex-enhanced immunoassay (The Binding Site, Birmingham, UK) on a Behring BNII auto-analyser (Dade Behring, Marburg, Germany) (166). Antibodies are directed against free light chain epitopes within the immunoglobulin molecule. The sensitivity of the assay is reported to be <5mg/L. Reference ranges were derived by examining sera of 100 healthy blood donors (Kappa: 11.38mg/L [95% CI: 7.41-16.77mg/l); Lambda: 17.36mg/L [95% CI: 8.91-29.87mg/L]).

Haematological response criteria

Haematological response was assessed as per international consensus criteria (167) (**Table 1.3**) at baseline and regular time-points thereafter as described in the methods section of each chapter. Patients who died prior to response assessment were classified as non-responders on an intention-to-treat basis.

Table 2.3: Haematological Response Criteria

Response Category	**Criteria**
Complete response	Negative serum and urine immunofixation and normal FLC ratio

Very good partial response	dFLC <40mg/L
Partial response	dFLC decrease >50%
Non-responder	dFLC decrease <50%

Cardiac Assessment

Biomarkers

The cardiac biomarkers, NT-proBNP and Troponin T, were measured in all patients at baseline and all subsequent follow up visits. Blood sample processing was performed by the laboratory at the Royal Free hospital. The resultant values were used to derive the Mayo stage for each patient. As per published guidance, the cut offs applied were: NT-proBNP >332ng/L and Troponin T >0.035mcg/L. Patients were allocated Stage I if both markers were below these thresholds, Stage II if one of these markers was above the threshold and Stage III if both markers were above the threshold (20). Furthermore, the European modification of the Mayo 2004 criteria was applied to patients with Stage III disease, to further subdivide them into IIIa and IIIb, based on an NT-proBNP threshold of >8500ng/L.

Echocardiography

All patients had an echocardiogram performed at baseline and each follow-up visit by three echocardiographers with expertise in amyloidosis. A GE Vivid E9 ultrasound machine equipped with a 5S probe was used to perform all echocardiograms. Measurements were performed offline using Echo PAC software (Version 202). The overall, basal and apical LS%, LV ejection fraction, LV wall thickness and markers of diastolic function (inclusive of EE' ratio) were performed and calculated in accordance with previously published guidance (168). Strain-

derived variables were acquired and calculated according to previous studies: septal longitudinal systolic apex to base (SAB) ratio (169) and relative apical longitudinal strain (RALS) as the average 4-chamber apical segments peak longitudinal strain/average basal and mid 4-chamber peak longitudinal strain (170).

In cases whereby echocardiogram was insufficient to exclude cardiac amyloidosis, cardiac magnetic resonance imaging was also performed. This method is highly sensitive for the detection of amyloid via the characteristic pattern of late gadolinium enhancement (171).

Organ Assessment

SAP scintigraphy

Patients underwent ^{123}I-SAP scintigraphy at baseline and follow up in order to assess and monitor visceral organ involvement. This investigation can visualise visceral amyloid deposits within the liver, kidney, spleen, adrenal glands and bones. An injection of 200µg of SAP with 190MBq of ^{123}I (equivalent of 3.8mSV of radiation) was administered by intravenous injection six or twenty-four hours prior to imaging. Subsequently, anterior, posterior and relevant oblique views were taken using a GE Starcam gamma camera (IGE Medical Systems, Slough, UK). The amyloid load was assigned to be normal, small, medium, large or equivocal as per the criteria set out in **Table 2.4**.

Table 2.4: Classification of amyloid burden on ^{123}I-SAP scintigraphy

Amyloid Burden	**Criteria**
Normal	No amyloid tracer localisation
Small	Tracer uptake in one or more organs without alteration in the intensity of tracer uptake in the blood pool
Medium	Tracer uptake in one of more organs with reduction in the intensity of tracer uptake in the blood pool
Large	Tracer uptake in one of more organs without evidence of tracer uptake in the blood pool
Progression	Increment between above categories
Regression	Decrement between above categories

Organ involvement and response criteria

Classification of visceral organ involvement at baseline was assigned via biopsy confirmation or as per international consensus criteria (167, 172) in the presence of biopsy proof of amyloid as an alternate site e.g. abdominal fat aspirate as outlined in **Table 2.5**. Soft tissue involvement was determined on the basis of either confirmatory histology or clinical evidence (e.g. macroglossia). Involvement of the spleen and adrenal glands was determined on the basis of tracer uptake on ^{123}I-SAP scintigraphy. Organ response criteria are also outlined in **Table 2.5**.

Table 2.5: Organ involvement and response criteria

Organ	Definition of involvement	Definition of response	Definition of progression
Heart	Mean left ventricular wall thickness of >12mm on echocardiogram in the absence of another cardiac cause	NT-proBNP reduction >30% and >300ng/L (assuming baseline ≥650ng/L) or improvement by 2 NYHA classes (assuming baseline class of 3-4)	NT-proBNP >30% and >300ng/L increase Troponin >33% increase Ejection fraction >10% decrease
Kidney	24-hour urinary protein >0.5g per day, predominantly albumin	50% decrease (≥0.5g/day) of 24h urinary protein (must be >0.5g/day at presentation) Creatinine and creatinine clearance must not worsen by 25% above baseline	50% increase (≥1.0g/day) of urine protein to >1g/day or 25% worsening of serum creatinine or creatinine clearance
Liver	Total liver span >15cm in the absence of heart failure or alkaline phosphatase >1.5 times the reference upper limit of normal	50% decrease in abnormal alkaline phosphatase	50% increase in alkaline phosphatase above the lowest value

		Decrease in liver size radiographically by at least 2cm	
Nerve	Peripheral: symmetric lower extremity sensorimotor peripheral neuropathy Autonomic: gastric-emptying disorder, pseudo-obstruction, voiding dysfunction not related to direct organ infiltration	Improvement in electromyogram nerve conduction velocity (rare)	Progressive neuropathy by electromyography or nerve conduction velocity

Quality of Life Assessment

All patients presenting to the NAC with suspected or confirmed AL amyloidosis were asked to complete a questionnaire, the short form 36 health survey questionnaire (SF36 Version 2 ® (173)). This questionnaire comprises of 36 questions across 8 health domains and was employed as a measure of self-reported outcomes in systemic AL amyloidosis. Within each domain, items are scored, coded, summed and transformed to a scale from 0 (worst possible health) to 100 (best possible health). On serial monitoring, a change in score of at least 10 within a given domain is deemed clinically significant. The domains covered by this questionnaire are:

1. Physical functioning (10 items) – covers the extent to which health impacts upon activities such as climbing stairs, carrying shopping bags and participation in sport. Low scores are indicative of significant limitations to physical functioning.
2. Social functioning (2 items) – covers the extent to which health impacts social activity such as visiting friends in the month prior to assessment. Low scores demonstrate a marked impact of health upon an individual's normal social activity.
3. Role limitations due to physical problems (4 items) – covers the impact of heath on usual daily activity such as attending work and completing housework. A low score indicates that the individual's health impacts upon work or other typical domestic activities of daily living.
4. Role limitations due to emotional problems (3 items) – covers the impact of health on usual daily social activity such as ability to achieve what one would like each day. A low score indicates a more marked impact of health upon usual daily activity secondary to emotional problems.

5. Mental health (5 items) – covers general psychological well-being inclusive of depression and anxiety within the month prior to assessment. Low scores indicate depressed or anxious the majority of the time.
6. Energy/Vitality (4 items) – covers fatigue and energy level. Low scores indicate the participant feels tired and lacks energy the majority of the time.
7. Pain (2 items) – covers the extent of pain in the four weeks prior to assessment. A low score indicates significant pain, which impacts usual activity.
8. General health perspectives (5 items) – covers an overall rating of general health. Low scores indicate poor perceived health.

Genetic Sequencing

All patients with a diagnosis of ATTR amyloidosis underwent genetic sequencing to exclude a heritable cause of the disease. Deoxyribonucleic acid was isolated, by a rapid method, from whole blood collected in an EDTA tube. Polymerase chain reaction was used to amplify the coding regions for the genes and appropriate exons. The primers used in the process are outlined in **Table 2.6**.

Table 2.6: Primers used for genotyping in patients with suspected hereditary amyloidosis

Gene	Exon	Primer sequence
Transthyretin	**2**	**Forward:** 5'-TTTCGCTCCAGATTTCTAATAC-3' **Reverse:** 5'-CAGATGATGTGAGCCTCTCTC-3'
	3	**Forward:** 5'-GGTGGGGGTGTATTACTTTGC-3' **Reverse:** 5'-TAGGACATTTCTGTGGTACAC-3'
	4	**Forward:** 5'-GGTGGTCAGTCATGTGTGTC-3' **Reverse:** 5'-TGGAAGGGACAATAAGGGAAT-3'
Apolipoprotein A-I	**3**	**Forward:** 5'-GGCAGAGGCAGCAGGTTTCTCAC-3' **Reverse:** 5'-CCAGACTGGCCGAGTCCTCACCTA-3'
	4	**Forward:** 5'-CACTGCACCTCCGCGGACA-3' **Reverse:** 5'-CTTCCCGGTGCTCAGAATAAACGTT-3'
Fibrinogen	**5**	**Forward:** 5'-GCTCTGTATCTGGTAGTACT-3' **Reverse:** 5'-ATCGGCTTCACTTCCGGC-3'

Statistical Analysis

Statistical analysis was performed using SPSS v25 (IBM SPSS0 and Graph Pad Prism (Version 50 software), Stata (Stata 2021. State Statistical Software: Release 17. College Station, Texas USA) or SAS/STAT software, Version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA) and R 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria, JM package, version 1.4-8).

The statistical analysis is described in detail within the methods section of each individual chapter.

Results Section 1:

Use of diagnostic screening biopsy and prognostic markers in amyloidosis

Chapter 3: The value of screening biopsy in AL and ATTR amyloidosis

This chapter is written in the context of my publication:

The value of screening biopsies in light-chain (AL) and transthyretin (ATTR) amyloidosis. Cohen OC, Sharpley F, Gilbertson JA, Wechalekar AD, Sachchithanantham S, Mahmood S, Whelan CJ, Martinez-Naharro A, Fontana M, Lachmann HJ, Hawkins PN, Gillmore JD. European Journal of Haematology (2020), 105(3), 352-356. Copyright permission obtained, from Wiley, as per copyright transfer agreement, for use in my thesis.

Introduction

Systemic amyloidosis is characterised by the accumulation of a misfolded protein within organs, leading to impairment of function. There is wide variability in presenting features leading to significant diagnostic delays – 72% of patients with AL amyloidosis are diagnosed at least 12 months from symptoms onset whilst around 80% visit at least 3 doctors prior to this point (174). Amyloidosis is a histological diagnosis that is typically established via biopsy of a clinically involved organ (175), which carries clinical risk, is costly (156, 176) and requires specific clinician expertise (177). Despite the high diagnostic specificity of non-biopsy diagnosis of cardiac transthyretin amyloidosis, as defined by Perugini grade 2/3 cardiac uptake on Tc-DPD scintigraphy in the absence of a monoclonal protein in serum and urine, up to 30% of patients will have a paraprotein or light chain imbalance, such that a tissue diagnosis is needed to exclude AL amyloidosis (64). This is typically an endomyocardial biopsy, which carries a risk of major complication (pericardial effusion, haemopericardium or

cardiac tamponade) in 0.2-0.7% of patients. This risk is highest in female patients undergoing the procedure in non-teaching hospitals (178).

Screening biopsies represent a low–risk approach to providing a histological diagnosis. The reported sensitivity of screening biopsies varies greatly between both amyloid subtypes (58) and biopsy sites: 75-96% for abdominal fat aspiration (58, 176, 179-182), 50-55% for bone marrow trephine and 50-70% for rectal biopsies (175). There is a paucity of evidence examining the diagnostic sensitivity and concordance of combining screening biopsies (182). The work up for AL amyloidosis routinely includes a diagnostic bone marrow trephine (156) and the high sensitivity of AFA in this setting, coupled with its procedural ease and safety (158), warrant its consideration as a routine adjunct to diagnosis. Additionally, there is limited data on the relationship between organ involvement and whole body amyloid burden in AL amyloidosis (58, 182). The diagnostic sensitivity of individual and combined screening biopsies together with the impact of amyloid load and visceral organ involvement upon this diagnostic sensitivity is reported here.

Method

All confirmed cases of AL or ATTR amyloidosis, seen at the UK NAC (2006-2019), who underwent both an AFA and either a bone marrow trephine or gastrointestinal biopsy at diagnosis were included. Confirmation of amyloid subtype was by immunohistochemistry or, in a minority of cases, mass spectrometry, except in patients who met the non-biopsy criteria for ATTR amyloidosis (64). All patients underwent a detailed baseline assessment inclusive of clonal and organ-specific blood markers, echocardiography and ^{123}I-SAP scintigraphy. Involvement of the heart, liver

and kidneys was determined as per international consensus criteria (172) whilst amyloid burden was determined by ^{123}I-SAP scintigraphy.

Abdominal fat aspiration was performed as previously described (158). Fat smears were formalin-fixed and double embedded in agar prior to production of a paraffin block. The block was sectioned and underwent Congo red staining and immunohistochemistry. Two experienced independent observers (a senior clinician and a senior laboratory scientist) blind to the clinical details, interpreted all slides. In the rare event of disagreement between the two observers, the case was discussed within a multi-disciplinary team meeting to reach consensus. Gastrointestinal and bone marrow biopsies were performed prospectively, prior to review at the NAC. The level of Congo red staining was graded as follows: 1 – single or very scant deposits; 2 – scanty deposits e.g. along 1-2 vessels; 3 – throughout selected areas; 4 – throughout the sample; 5 – complete replacement of normal tissue architecture by sheets of amyloid.

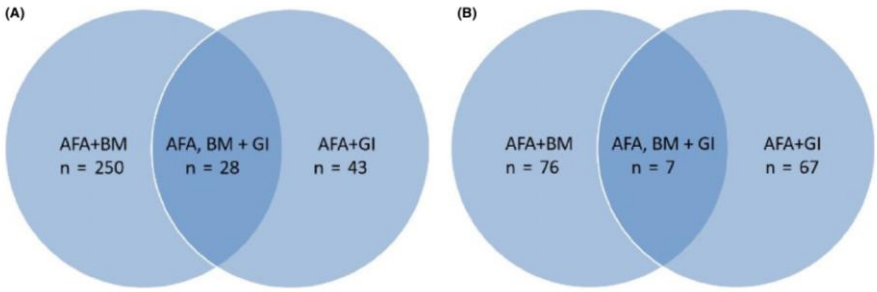
Statistical analysis was performed using SPSS version 25. All p values were 2-sided with a value of <0.05 considered significant. Approval for analysis and publication was obtained from the Royal Free hospital institutional review board and all patients, via written consent forms, in accordance with the Declaration of Helsinki.

Results

Four hundred and seventy one patients were identified (AL: n=321; ATTR: n=150). The number of biopsies included in patients with both AL and ATTR amyloidosis is displayed in **Figure 3.1**. Patients had a median age of 75 (41-95) years whilst 338 (71.6%) patients were male. Within patients diagnosed with AL amyloidosis,

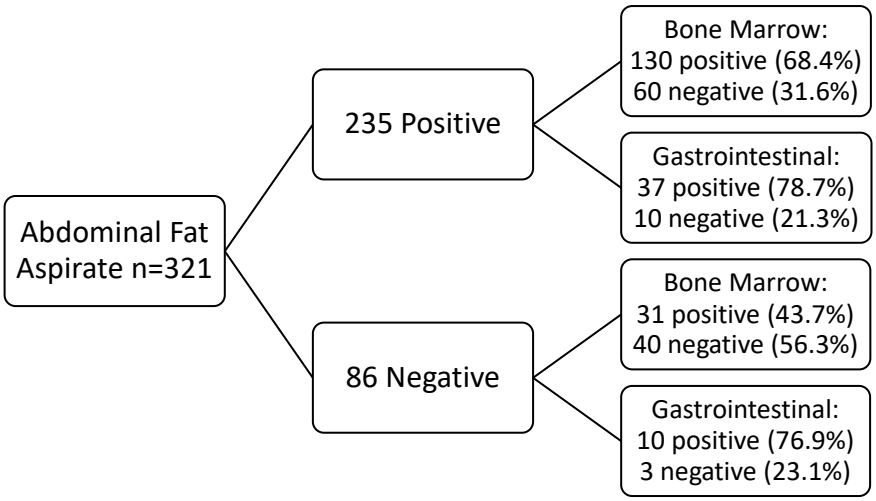
231/321 (72.0%) had lambda-secreting clones. Amyloidotic organ involvement was: heart – 68.8%, kidneys – 43.9% and liver – 19.0%.

Figure 3.1: Number of biopsies included in patients with (A) AL and (B) ATTR amyloidosis



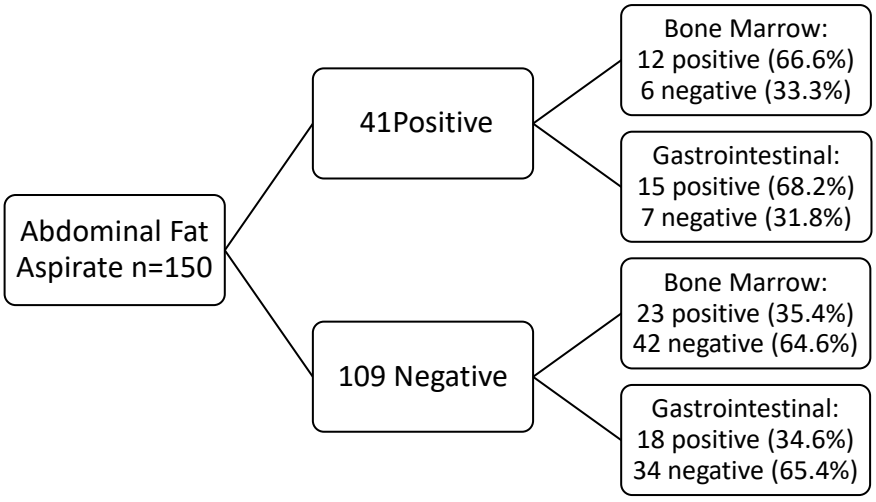
In total, 235/321 (73.2%) fat aspirates were positive for amyloid in patients with systemic AL amyloidosis. There was no association between the presence of amyloid on AFA and light-chain immunoglobulin isotype (p=0.158). Furthermore, in patients with AL amyloidosis, 166/278 (59.7%) bone marrow trephines and 53/71 (74.6%) gastrointestinal biopsies were positive for amyloid. Gastrointestinal biopsy sites were: 36 (50.7%) rectal, 18 (25.4%) colon, 9 (12.7%) stomach, 6 (8.5%) duodenal and 2 (2.8%) oesophageal. In terms of concordance, patients who had amyloid confirmed on AFA and underwent either a BMT (n=195) or gastrointestinal (n=50) biopsy, had amyloid detected on this second biopsy in 67.2% and 78.0% of cases respectively. Amongst the 86 AL amyloid patients in whom AFA failed to detect amyloid, 47.1% of cases were identified by an alternate screening biopsy (35 BMT, 14 gastrointestinal) (**Figure 3.2**).

Figure 3.2: Diagnostic sensitivity and concordance of screening biopsy in AL amyloidosis.



The diagnostic sensitivity of screening biopsies in ATTR amyloidosis was significantly lower than in AL amyloidosis (AFA: 27.3%, p=0.0001; BMT: 41.4%, p=0.0007; Gastrointestinal: 44.6%, p=0.0001). Furthermore, diagnostic sensitivity was lower in patients with wild-type ATTR amyloidosis (n=117, sensitivity: 16%) than those with hereditary ATTR amyloidosis (n=38, sensitivity: 61%) (p=0.0001). There appeared to be a similar, yet non-significant trend in both bone marrow (57% vs. 38%) and gastrointestinal (50% vs. 42%) biopsies. When ATTR amyloid was present on AFA, it was also identified in 66.6% of BMTs and 68.2% of gastrointestinal biopsies. Conversely, ATTR deposits were found in 35.4% BMTs and 33.3% of gastrointestinal biopsies when AFA did not detect amyloid (**Figure 3.3**).

Figure 3.3: The diagnostic sensitivity and concordance of screening biopsy in ATTR amyloidosis.



The grade of amyloidosis on Congo red staining by biopsy site and amyloid subtype is documented in **Table 3.1**. Across both AL and ATTR amyloidosis, Congo red stain grading was higher in AFA than BMT biopsies ($p<0.0001$ and $p=0.05$) respectively. In AL amyloidosis, Congo red grading was also higher in AFA than gastrointestinal biopsies ($p<0.0001$) but this finding was not replicated in ATTR amyloid ($p=0.21$). There was no significant difference in gastrointestinal biopsy sensitivity based on whether the clinical indication was purely for screening or related to investigation of symptoms ($p=0.26$).

Table 3.1: Histological grading of amyloid based upon Congo red straining by amyloid subtype and biopsy site.

	AL amyloidosis			ATTR amyloidosis		
Grading	AFA (n,%)	BM (n,%)	GI (n,%)	AFA (n,%)	BM (n,%)	GI (n,%)
1	30 (12.8)	59 (35.5)	8 (15.1)	8 (19.5)	14 (40.0)	10 (30.3)
2	80 (34.0)	62 (37.3)	13 (24.5)	11 (26.8)	10 (28.6)	10 (30.3)
3	83 (35.3)	43 (25.9)	22 (41.5)	15 (36.6)	10 (28.6)	10 (30.3)
4	26 (11.1)	2 (1.2)	1 (1.9)	4 (9.8)	1 (2.9)	2 (6.1)
5	14 (6.0)	0 (0)	5 (9.4)	1 (2.4)	0 (0)	0 (0)
Insufficient tissue	2 (0.9)	0 (0)	1 (1.9)	2 (4.9)	0 (0)	1 (3.0)
Total	235	166	53	41	35	33

In systemic AL amyloidosis, diagnostic sensitivity of screening biopsies was associated with organ involvement (**Table 3.2**). Patients with liver involvement were significantly more likely to have amyloid detected on both AFA (91.8% vs. 69.0%; p=0.0002) and gastrointestinal biopsy (100.0% vs. 71.4%; p=0.04). Sensitivity of bone marrow trephine biopsies was similar (71.4% vs. 69.5%; p=0.10). In patients with cardiac and renal involvement, the diagnostic sensitivity of abdominal fat aspiration was similar (cardiac: 71.4%, renal: 69.5%; p=0.898) as was the case for other screening biopsies.

Table 3.2: Diagnostic sensitivity of screening biopsies by organ involvement

	Cardiac Involvement		Hepatic Involvement		Renal Involvement		No visceral involvement	
	N	Positive	N	Positive	N	Positive	N	Positive
Fat	221	170 (76.9%)	61	56 (91.8%)	141	110 (78.0%)	48	31 (64.6%)
BM	190	120 (63.2%)	49	35 (71.4%)	122	84 (68.9%)	37	15 (40.5%)
GI	45	35 (77.8%)	12	12 (100.0%)	21	17 (81.0%)	9	7 (77.8%)

The combination of AFA and BMT, was associated with an overall diagnostic sensitivity in systemic AL amyloidosis of 82.9% for all patients rising to 86.3% in patients with visceral organ involvement (85%, 96% and 87% in patients with cardiac, hepatic and renal amyloidosis respectively). Patients without clinically important visceral organ involvement (n=48) had a diagnostic sensitivity for AFA, BMT and gastrointestinal biopsies of 64.6%, 40.5% and 77.8% respectively. The combination of AFA and BMT had a diagnostic sensitivity of 71.4%.

Amyloid load, defined by ^{123}I-SAP scintigraphy, was strongly associated with diagnostic sensitivity of screening biopsies in systemic AL amyloidosis, although even amongst patients without visceral amyloid deposits, the diagnostic sensitivity of both AFA (69.0%) and gastrointestinal biopsy (68.0%) was over two thirds (**Table 3.3**).

Table 3.3: Diagnostic sensitivity of screening biopsies by amyloid load

Amyloid load N = 316	AFA positive	AFA Diagnostic sensitivity	BMT positive	BMT Diagnostic Sensitivity	GI biopsy positive	GI Biopsy Diagnostic Sensitivity	Total n (%)
Large	38/42	90.5%	28/35	80.0%	9/10	90.0%	42
Moderate	34/42	81.0%	27/37	73.0%	4/5	80.0%	42
Small	50/74	67.6%	35/54	64.8%	17/22	77.3%	74
None	109/158	69.0%	76/141	53.9%	17/25	68.0%	158

Discussion

This study highlights the value of screening biopsies to establish a diagnosis, particularly in AL amyloidosis, without the need for more invasive, higher risk, higher cost target organ biopsies. In AL amyloidosis, the diagnostic sensitivity of AFA was 73.2%, rising to 82.9% in combination with BMT, which is comparable to the 89% sensitivity of combining these procedures reported previously (182). Of note, these two studies utilised specialist amyloid centres to process and report the samples thereby minimising the risks of high inter-observer variability and false negatives,

which have historically posed a problem (183). This underlines the value of central review of screening biopsy to maximise diagnostic sensitivity. The combination of AFA and BMT left just 17.1% of patients requiring visceral organ biopsy to establish a histological diagnosis.

Screening biopsies are more likely to be positive if amyloid burden is high and if there is critical organ (cardiac, hepatic, renal) involvement by amyloid. The high diagnostic sensitivity in patients with hepatic amyloidosis likely reflects the fact that liver amyloid is invariably associated with the presence of amyloid in other organs (184), and typically indicates a high overall disease burden. Patients with cardiac, renal or hepatic amyloid represent the group most likely to be referred for target organ biopsy. This cohort had a combined diagnostic sensitivity of 86.3% (compared to 62.4% with BMT alone) leaving just 13.7% requiring a 'higher risk' critical organ biopsy. The majority of other patients with AL amyloidosis had predominant soft tissue involvement (e.g. macroglossia), which is more easily amenable to tissue sampling. Consequently, we would advocate performing AFA at the same time as BMT, which is routinely undertaken to ascertain plasma cell percentage (156), in cases of suspected AL amyloidosis.

In ATTR amyloidosis, the need to establish a definitive histological diagnosis in patients' who do not meet non-biopsy diagnostic criteria (64) has increased with the availability of new gene silencing medications and clinical trials of novel therapeutics, access to which require a firm diagnosis. Whilst the diagnostic sensitivity of AFA, BMT and gastrointestinal biopsies is considerably lower in ATTR amyloidosis, frequent cardiac involvement and the significant, albeit low, mortality and serious complication risk associated with endomyocardial biopsy, quoted as ~6% in most studies (183), nonetheless encourages their initial use.

In summary, AFA is a simple, low-risk procedure that can be performed at the bedside at the time of BMT in patients with suspected AL amyloidosis. The data suggests that this combination of procedures should be introduced as standard practice to minimise the need for more invasive, higher risk target organ biopsies. Screening biopsy review in a specialist amyloidosis centre leaves just 17.1% patients with AL amyloidosis requiring target organ biopsy. The sensitivity of screening biopsies in ATTR amyloidosis remains poor.

Chapter 4: The impact of longitudinal strain on haematological and cardiac response and survival in systemic AL amyloidosis

This chapter is written in the context of my publication:

Longitudinal Strain is an independent predictor of survival and response in patients with systemic AL amyloidosis. OC Cohen, A Ismael, B Pawarova, R Manwani, S Ravichandran, S Law, D Foard, A Petrie, S Ward, B Douglas, A Martinez-Naharro, L Chacko, CC Quarta, S Mahmood, S Sachchithanantham, HJ Lachmann, PN Hawkins, JD. Gillmore, M Fontana, RH Falk, CJ Whelan and AD Wechalekar. European Heart Journal. 4(21): 333-341. Permission obtained from the journal for use in this thesis.

Introduction

Cardiac involvement is common in AL amyloidosis, seen in around 75% of cases (23). The process is characterised by the deposition of amyloidogenic light chains, produced by a plasma (or B) cell clone, in the myocardium leading to damage (185). The diagnosis of cardiac involvement is based upon imaging, typically by echocardiogram, as per international consensus criteria (172). This method is relatively non-discriminatory given the potential for other comorbidities to contribute to an increased left ventricular wall thickness (172). Conversely, it is NT-proBNP and Troponin that define Mayo stage and that are used in the assessment of treatment response (20, 167). Again, these factors are subject to interference from other co-morbid states, particularly in patients with major fluid shifts as seen in dialysis-dependent individuals. Despite that, patients with an NT-proBNP >8500ng/L have

been shown to have especially poor outcomes with a median survival of just 3 months (22).

A far more sensitive marker of cardiac amyloidosis, the loss of longitudinal strain to determine loss of longitudinal cardiac function, can also be determined by echocardiography. Associated relative apical sparing of longitudinal strain impairment is particularly specific for amyloidosis (186). A meta-analysis concluded that the normal range for longitudinal strain was deemed to be between -20.9% to -27.8% across 24 studies (187). Less negative strain (i.e. closer to zero) equates to worse systolic left ventricular function. This marker, along with others such as myocardial contraction fraction (188), stroke volume index (188), E/e' and LV mass (189), predicts survival. However, only longitudinal strain is widely used in this setting. Despite this, larger studies examining LS% in a population of patients with AL amyloidosis treated uniformly with upfront bortezomib are sparse within the literature.

The aim of chemotherapy in AL amyloidosis is to suppress the malignant clone and thus prevent the production of amyloidogenic light chains and their resultant tissue damage (185). Following suppression of the clone, monitoring for improvement in cardiac function is conducted via NT-proBNP measurements, which reflect mechanical stress on cardiac myocytes by the direct proteotoxic effect of the light chain oligomers but, as stated, are also incredibly sensitive to alterations in fluid balance (190). These confounding factors, such as fluid balance, have far less impact on longitudinal strain. Longitudinal strain has been shown to correlate with NT-proBNP response and improve after successful treatment of AL amyloidosis (191). Here, we report the impact of longitudinal strain at baseline and following treatment in a large cohort of bortezomib-treated newly diagnosed patients with systemic AL amyloidosis with cardiac involvement.

Method

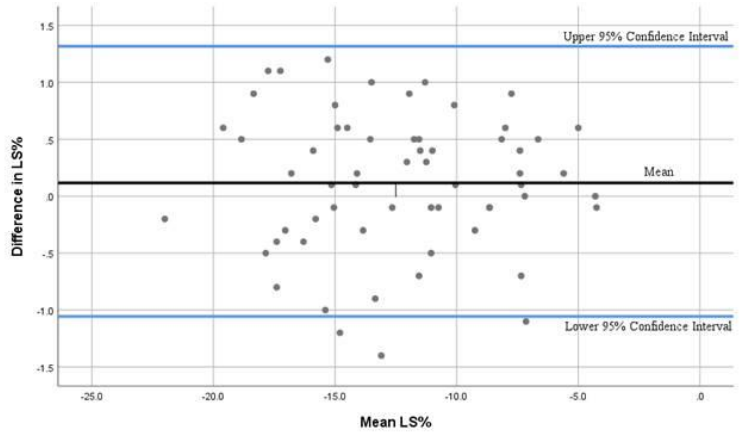
All newly diagnosed patients with AL amyloidosis, who enrolled in a prospective observational study (ALchemy) at the UK National Amyloidosis Centre (2010-2017), were included. All patients had a diagnosis of amyloidosis confirmed via central review of histological material with amyloid subtype determined by immunohistochemistry or mass spectrometry. All patients had a full baseline evaluation inclusive of blood monitoring of organ function and echocardiography and were treated with bortezomib first line. Patients were stratified by the European modification of the 2004 Mayo stage (Mayo stage I, Mayo Stage II whilst Mayo stage III patients were subdivided into IIIa [NT-proBNP<8500ng/L] and IIIb [NT-proBNP≥8500ng/L](22)). Furthermore, baseline echocardiogram was used to stratify patients into quartiles by LS%. Organ involvement, organ response and haematological response were determined by international consensus criteria (167, 172, 192). Patients who had cardiac involvement by CMR (based on review at a central multidisciplinary meeting) were also included irrespective of left ventricular wall thickness. In additional to traditional haematological response, a dFLC <10mg/L (as published previously (104)) was also evaluated. Overall survival was calculated from date of diagnosis to death from any cause.

Echocardiograms were performed using a GE Vivid E9 ultrasound machine equipped with a 5S probe and measurements performed offline using Echo PAC software (Version 202). The overall, basal and apical LS%, LV ejection fraction and LV wall thickness were performed and calculated in accordance with previously published guidance (168). In order to ensure consistency, all echocardiograms was analysed by a single experienced operator. This operator was later asked to repeat a sample of 10% of echocardiograms to evaluate intra-observer variability whilst a second sample of 10% of echocardiograms were analysed by a second operator to

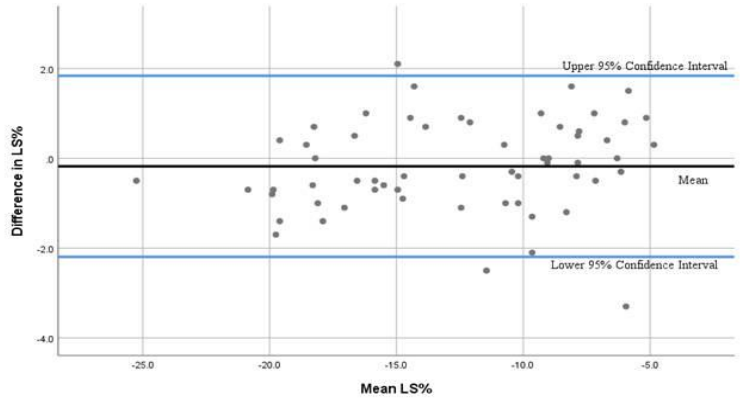
assess inter-observer variability. The operators were blinded to prior measurements. The mean difference in calculated LS% was 0.12% (95% CI: -0.16% – +1.32%) and 0.18% (-2.18% - +1.82%) for intra and inter-observer variability respectively (**Figure 4.1** displays the respective Bland-Altman plots).

Figure 4.1: Intra (A) and Inter (B) observer variability in LS% measurements

A)



B)



Statistical analysis was performed using IBM SPSS version 25. Approval for analysis and publication was obtained from the National Health Service institutional review board. Written consent was obtained from all patients in accordance with the Declaration of Helsinki. The Kaplan-Meier method was used to analyse survival outcomes. Multivariable modelling by Cox regression analysis was performed to assess the impact of Mayo stage and LS% on survival. Two-tailed unpaired t-tests were used to compare two continuous variables whilst analysis of variance (ANOVA) was used when >2 continuous variables were compared. All p-values were 2-sided with a significance level of <0.05.

Results

A total of nine-hundred and fifteen patients were included within the study. **Table 4.1** displays the baseline characteristics whilst **Figure 4.2** shows the number of patients evaluable at each time point. Overall longitudinal strain was independent of regional LS% in predicting survival (Hazard ratio 1.17 [1.03-1.33], p=0.02 for overall LS% whilst p>0.05 in baso-lateral, baso-septal, apico-lateral and apico-septal strain measurements). Additionally, overall longitudinal strain percentage was independent of other demographic factors (age, sex) and cardiovascular comorbidity in predicting survival within a multivariable analysis (**Table 4.2**). Six-hundred and twenty eight (68.6%) patients had cardiac involvement with a mean baseline LS% of -12.7% (compared to -15.1% in the cohort overall). The LS% worsened with Mayo stage (Mayo Stage I: -21.1%, Mayo Stage II: -17.1%, Mayo Stage IIIa: -12.9% and Mayo Stage IIIb: -12.1% [p<0.0001]) as shown in **Figure 4.3**. Finally, in patients with cardiac involvement, those who died within 6 months of diagnosis had a median longitudinal strain % of -10.2% compared to -13.8% in those surviving longer (p<0.0001).

Table 4.1: Baseline patient characteristics by cardiac involvement

Median (range) / n (%)	Cardiac Involvement (n=628)	No Cardiac Involvement (n=287)
Age (years)	68 (32-89)	71 (45-92)
Male	61.1%	46.0%
NYHA class: 1 2 3 4 Not recorded	 99 (15.8) 333 (53.0) 98 (15.6) 3 (0.5) 95 (15.1)	 124 (44.3) 113 (39.4) 10 (3.5) 1 (0.4) 39 (13.6)
NT-proBNP (ng/L) High-sensitivity Troponin (ng/L)	4076 (93-93796) 78.5 (3-527)	329 (12-19315) 19 (1-176)
Co-morbidity: Diabetes mellitus Ischaemic Heart Disease Valvular disease Atrial fibrillation Pre-existing hypertension	 42 (6.8) 71 (11.5) 91 (14.8) 77 (12.5) 140 (22.7)	 n/a
Mayo Stage: I II IIIa, NT-proBNP ≤8500 ng/L IIIb, NT-proBNP >8500 ng/L Not recorded	 7 (1.1) 189 (30.1) 301 (47.9) 117 (18.6) 14 (2.2)	 133 (46.3) 105 (36.6) 33 (11.5) 5 (1.7) 11 (3.8)
Median SBP (mmHg)	116 (76-194)	130 (88-190)
Renal involvement No. patients on dialysis	378 (60.2) 24 (3.8)	245 (85.4) 24 (8.4%)
No. organs involved	2 (1-5)	1 (1-3)
dFLC (mg/L)	236 (2.5-15898)	105 (0-3822)

Figure 4.2: Flow diagram demonstrating the number of evaluable patients at each time point

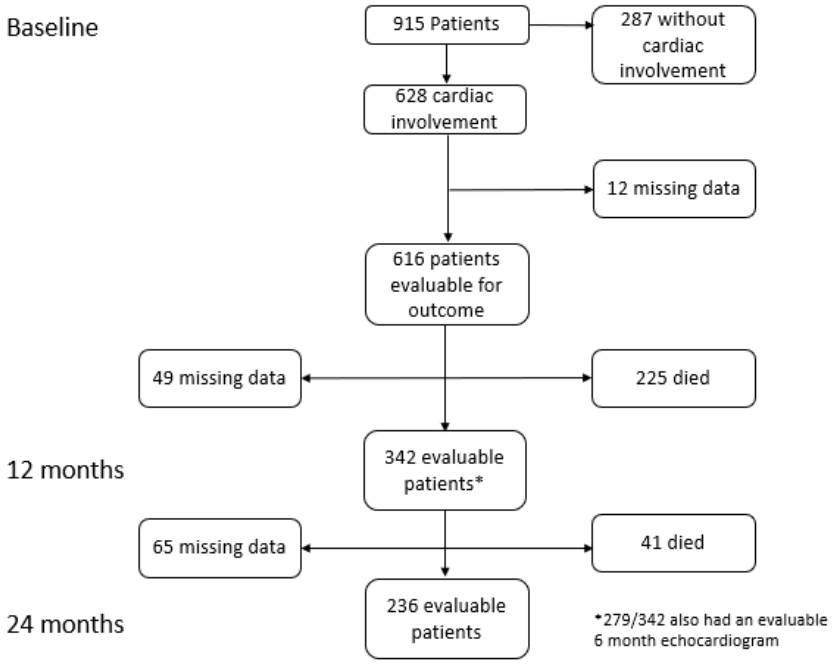
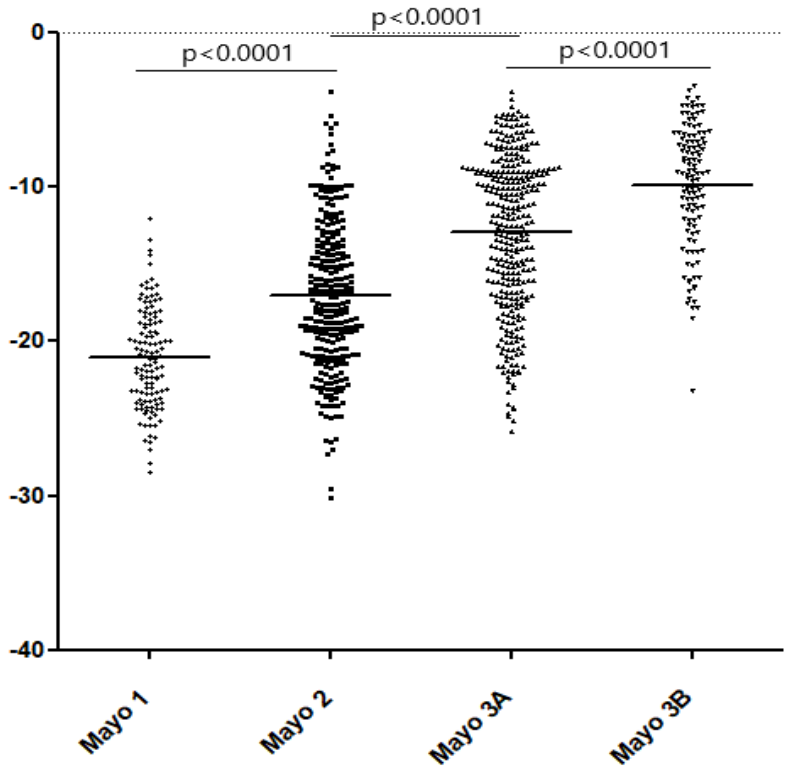


Table 4.2: Multivariable model examining patient demographics, comorbidities and longitudinal strain in predicting overall survival

	Wald	Hazard ratio	95% CI	Significance
Age	0.78	1.01	0.99-1.03	0.38
Atrial Fibrillation	1.97	1.57	0.84-2.96	0.16
Creatinine	1.83	1.00	1.00-1.03	0.18
Diabetes	3.81	0.51	0.99-3.87	0.051
Hypertension	0.01	1.02	0.64-1.65	0.92
Ischaemic Heart Disease	1.48	1.49	0.78-2.82	0.22
Longitudinal strain	11.3	1.07	1.03-1.18	0.001
Sex	1.24	0.88	0.71-1.10	0.27
Valvular disease	0.08	1.06	0.70-1.61	0.78

Figure 4.3: Baseline longitudinal strain by cardiac Mayo stage



The median OS of the whole cohort was 61 (95% CI: 49.9-72.1) months (**Figure 4.4**). By Mayo stage, OS was: Mayo I and II: not reached, Mayo IIIa: 30 (95% CI: 23.1-36.9) months and Mayo IIIb: 4 (95% CI: 1.8-6.2) months. Whilst in patients with cardiac involvement, the OS was 31 (95% CI: 23.9-38.1) months. Patients were stratified by LS% into quartiles. Overall survival worsened significantly with worsening LS% category (LS% ≤ -16.2%: 80 months, -16.1% - -12.2%: 36 (95%CI:20.9-51.1) months, -12.1% - -9.1%: 22 (95%CI:9.1-34.9) months and ≥ -9.0%: 5 (95%CI:3.2-6.8) months (p<0.0001) (**Figure 4.5**).

Figure 4.4: Overall survival of all patients by cardiac involvement

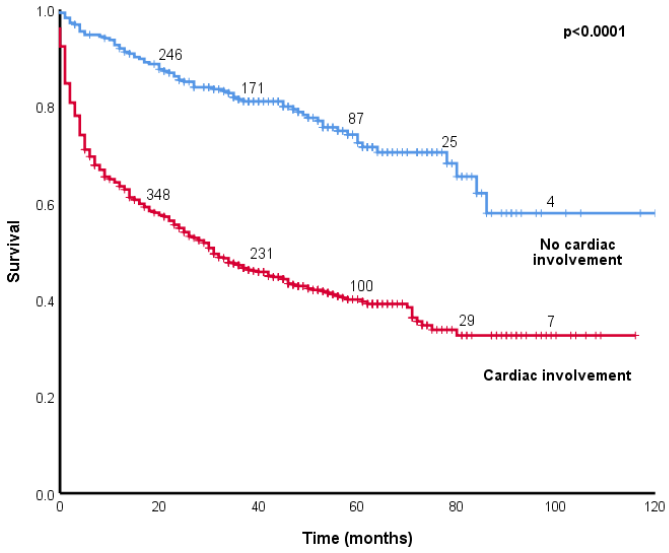
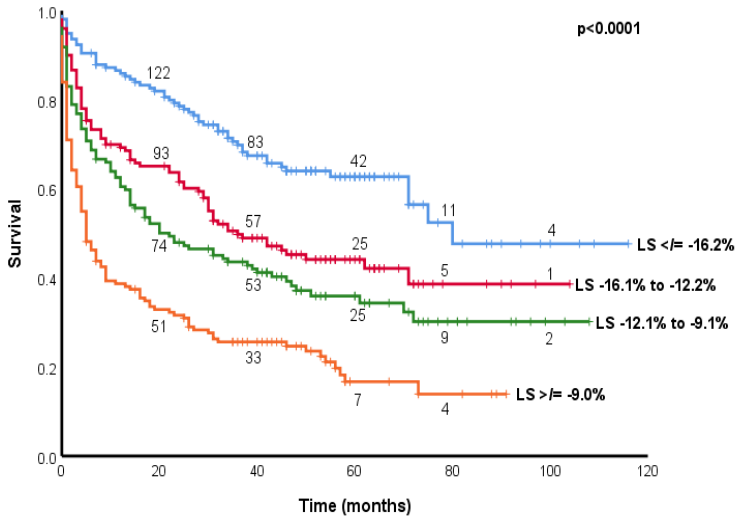


Figure 4.5: Overall survival of patients with cardiac AL amyloidosis stratified by LS% quartile: LS% ≤ -16.2%, -16.1% - -12.2%, -12.1% - -9.1% and ≥ -9.0%.



On univariate analysis, the cardiac biomarkers, NT-proBNP (Hazard ratio 2.58 [95%CI:2.09-3.19], p<0.0001) and Troponin T (Hazard ratio 3.61 [95%CI:2.56-5.08], p<0.0001), predicted survival. On multivariable analysis, the lower LS% cut-offs (-12.1% - -9.1%, ≥-9.0%) were independent of cardiac Mayo staging, systolic blood pressure, ejection fraction and LV wall thickness in predicting survival (**Table 4.3**).

Table 4.3: Multivariable analysis examining the impact of longitudinal strain, Mayo stage, Ejection fraction, left ventricular wall thickness and systolic blood pressure on survival

	Hazard ratio	95% CI	P value
LS% ≤ -16.2%	Reference		
-16.1% and -12.2%	1.44	0.98-2.11	0.06
-12.1% and -9.1%	1.60	1.08-2.36	0.019
≥ -9.0%	2.24	1.49-3.36	<0.0001
Mayo stage			
II	1.37	0.43-4.39	0.59
IIIa	1.33	0.42-4.19	0.63
IIIb	2.02	0.63-6.46	0.24
Ejection fraction	1.21	0.94-1.55	0.13
LV wall thickness	0.92	0.53-1.61	0.78
Systolic blood pressure <100mmHg	1.01	0.76-1.34	0.96

The impact of a change in LS% on survival

Strain was evaluable at both study onset and 12 months in 342 patients. Serial change in strain cut-offs were assessed between 0.5% and 3.0%; all of which significantly improved overall survival (**Table 4.4**). However, despite statistical significance, there is no robust data or prior publication to guide the level of strain improvement that should be deemed clinically significant. It was determined that an improvement in longitudinal strain of 2.0% would be the minimum value to account for inter-observer variability and likely represent a clinically meaningful change in strain. This was deemed an 'LS-response.' Patients who achieved an 'LS-response' did have a worse strain at baseline (-12.9% vs. -14.4%) whilst other demographics, Mayo stage and cardiac comorbidities were comparable between groups (**Table 4.5**)

Table 4.4: Impact of serial longitudinal strain % cut-offs on survival in patients with cardiac AL amyloidosis

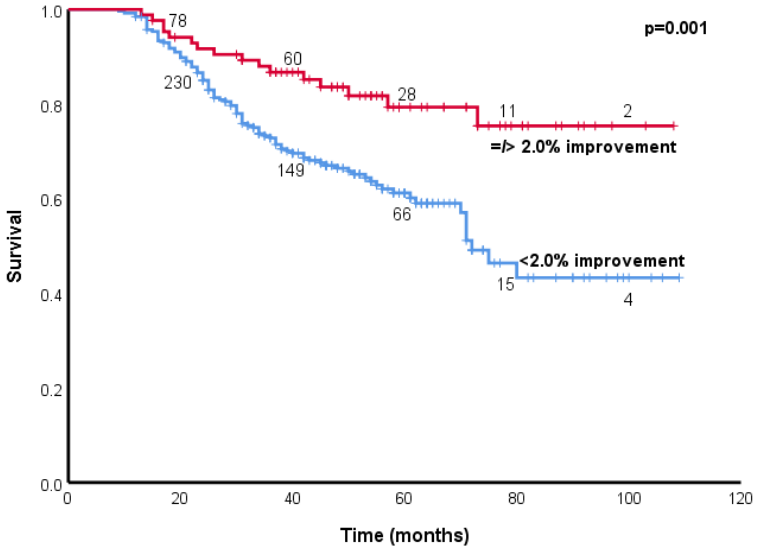
LS% absolute improvement	% of evaluable patients with LS% improvement	Overall Survival of patients achieving this level of LS% response vs. lesser LS% improvement/worsening.	P value
Any improvement	161/342 (47.1%)	NR vs. 72 (95% CI: 57.0-87.0) months	P=0.007
-0.5%	141/342 (41.2%)	NR vs 71 (95% CI: 67.0-75.0) months	P=0.008
-1.0%	124/342 (36.3%)	NR vs. 71 (95% CI: 67.0-75.0) months	P=0.002
-1.5%	101/342 (29.5%)	NR vs. 72 (95% CI: 65.1-78.9) months	P=0.003
-2.0%	85/342 (24.9%)	NR vs. 72 (95% CI: 64.8-79.2) months	P=0.001
-2.5%	68/342 (19.9%)	NR v 75 (95% CI: 67.4-78.6) months	P=0.002
-3.0%	58/342 (17.0%)	NR v NR	P=0.024

Table 4.5: Baseline patient characteristics by LS% strain response (inclusive of patients with cardiac AL amyloidosis only)

	Patients achieving a 2% improvement in LS% at 12 months (n=85)	Patients not achieving a 2% improvement in LS% at 12 months (n=257)	P value
Age	67 (32-88)	67 (40-87)	0.66
Sex	39 (45.9%) male 46 (54.1%) female	93 male 164 female	0.13
Baseline LS%	-12.9% (-22.1% - -3.7%)	-14.4% (-27 - -3.8)	0.0001
Baseline ejection fraction	56.2% (34-70)	56.6% (21-75)	0.68
Baseline LV wall thickness	14.6cm (9-20)	15.0cm (9-25)	0.18
Mayo stage	I 2 II 34 IIIa 34 IIIb 13	I 3 II 92 IIIa 133 IIIb 24 Not recorded. 5	0.93
Diabetes	5	15	0.98
Ischaemic heart disease	5	22	0.43
Valvular disease	28	63	0.13
Hypertension	14	54	0.37
Creatinine	102 (32-223)	128.5 (39-302)	0.06
Systolic blood pressure	118 (80-165)	119 (86-184)	0.77
NT-proBNP	5463 (229-33872)	5128 (93-93796)	0.90

The OS at 50 months was not reached in patients who achieved a 2.0% LS% improvement at 12 months compared to 72.0 (95% CI: 64.8-79.2) months in those not achieving this 2.0% improvement (p=0.001) (**Figure 4.6**). Notably, this effect persisted at 24 months with patients whose LS% had changed by -2.0% or more living longer (not reached at 63 months vs. 72 [95%CI:65.2-78.8] months, p<0.0001))

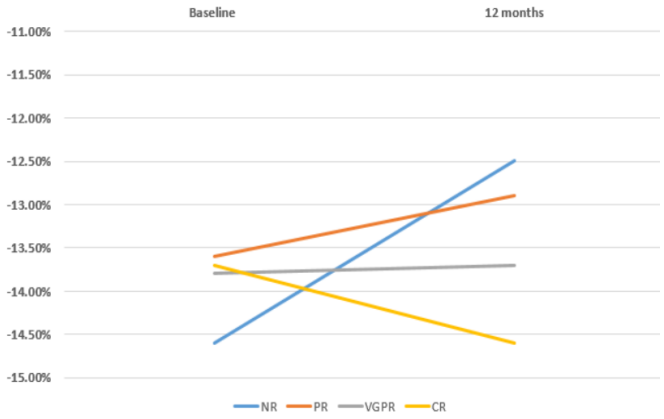
Figure 4.6: Overall survival of patients with cardiac AL amyloidosis by LS% improvement



The impact of haematological and cardiac organ response on LS%

Of the 342 patients with evaluable echocardiograms to assess LS% at baseline and 12 months, 325 patients also had evaluable haematological response assessments. In this patient group, the haematological responses were: CR – 82 (25.2%), VGPR – 143 (44.0%), PR – 71 (21.8%) and NR – 29 (8.9%). Patients in a complete haematological response showed improvement in longitudinal strain whilst strain worsened in patients in a lesser haematological response (**Figure 4.7**). No improvement was seen at 6 months irrespective of haematological response but worsening did occur, particularly in haematological non-responders (-14.6% to -12.4%, p=0.0005).

Figure 4.7: Change in longitudinal strain between baseline and 12 months according to haematological response

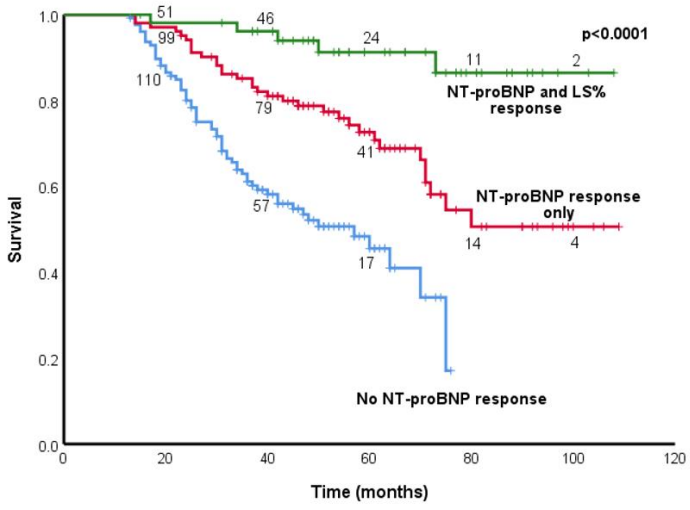


Twenty six (31.7%) patients in a complete haematological response also achieved a LS-response at 12 months. Patients who achieved both a complete haematological response and LS-response lived longer than those achieving a CR alone (median OS not reached in either category, p=0.009). Patients whose CR persisted to 24 months had an improvement in LS% from median -13.5% at baseline to -15.7% at 24 months (n=44; p=0.0002) with 49.2% achieving an LS-response. Conversely, 12.5% patients in a complete haematological response who progressed prior to the 24-month time point were in a CR (p=0.0001). Recent publications have highlighted the value of deeper haematological responses to optimise overall survival (104). Of patients in a complete haematological response, those patients also achieving a dFLC<10mg/L (n=70) demonstrated a median improvement of -1.1% in

LS% (p=0.02). Patients achieving a CR without a dFLC <10mg/L did not significantly improve their LS%.

Finally, the change in LS% was evaluated in relation to the biomarker-defined cardiac organ response as per international consensus criteria. Of evaluable patients with available assessments at baseline, 12 and 24 months, 65.5% (133/203) achieved a cardiac organ response. Of cardiac biomarker responders, 36.9% met criteria for an LS-response with a highly significant improvement in median longitudinal strain % (from -12.8% to -14.4%, p<0.0001). The LS% deteriorated (-14.4% to-13.4%, p=0.004) in cardiac biomarker non-responders. Patients achieving an LS-response in addition to a cardiac biomarker response survived longer than those meeting criteria for a cardiac organ response alone (p=0.0001; **Figure 4.8**). The same pattern was observed at 24 month (p<0.0001).

Figure 4.8: Overall survival by attainment of cardiac biomarker and longitudinal strain percentage response



Discussion

This study confirms the prognostic value of LS% at baseline in patients with cardiac AL amyloidosis. Critically, the importance of LS% to monitor response to treatment at 12 months and assess improvement in cardiac function is also demonstrated. The longitudinal strain improves significantly in patients achieving deep haematological responses. A -2.0% improvement was deemed clinically meaningful at 12 and 24 months and such an improvement prolongs long-term survival. Based on this data, new staging and response assessment criteria are postulated.

Longitudinal strain provides incremental survival data over cardiac stage and other echocardiographic parameters (193). A prior smaller study associated a strain value of -10.2% (191) at baseline with particularly poor outcomes (in our series, mean LS% of cardiac AL patients who died within 6 months was also -10.2%). This study demonstrates that stratification by baseline LS% identifies clear prognostic groups with OS best in the higher LS% category (≤-16.2%). A strain ≥-12.1% provided incremental survival data over other echocardiographic parameters (LV wall thickness and ejection fraction) and Mayo stage criteria inclusive of the Mayo IIIb poor prognostic group. Very few variables have previously been shown to be independently prognostic over and above NT-proBNP >8500ng/L, which defines the Mayo IIIb category.

Whilst monitoring of haematological response in AL amyloidosis is well established, the evaluation of change in function of affected organs remains sub-optimal. This study demonstrates that the LS% does not improve in the first 6 months in the majority of patients. This may be explained by the fact that there is ongoing amyloid deposition during induction chemotherapy, which typically lasts 6 months, until a deep haematological response is achieved. Additionally, amyloid fibrils are typically

resistant to degradation and are only cleared once the amyloidotic precursor is suppressed (194). Subsequently, at 12 months, improvement in LS% is almost exclusively seen in patients achieving a complete haematological response.

We attempted to define an absolute value that would represent a clinically meaningful, reproducible improvement in LS%. There is no data to guide this for amyloidosis. There are limited prior studies, which have demonstrated the value of LS% recovery in the post-myocardial infarct setting (195)) but no degree of improvement was specified. This study demonstrates that any cut off used to define LS% improvement conferred a survival advantage. We deemed that a -2.0% improvement represented the minimum value that was likely indicative of a clinically meaningful improvement; termed an “LS-response”. Furthermore, this value accounted for the relatively wide variation across individual measurements and was close to the 95% confidence interval for inter-observer variability, which had the widest variance. The LS-response identified patients with improved survival at both 12 and 24 months over and above those patients who had achieved a traditional cardiac biomarker-based response. This LS% improvement likely reflects true amyloid clearance from the LV whilst the cardiac response, measured by improvement in NT-proBNP, is less specific but likely reflects the lack of ongoing proteotoxic damage to cardiac myocytes. Critically, a poor baseline LS% did not preclude a cardiac response. Patients achieving a LS% response did have a poorer LS% at baseline, which may have, in part, occurred due to selection bias in that patients had to be alive at 12 months to be included in the change in LS% analysis. Patients with a poor LS% who failed to respond to therapy would be more likely to die prior to this time point. These data support the consideration of absolute improvement in LS% as an additional criterion for cardiac response in AL amyloidosis. We propose a model that

incorporates strain along with usual biomarker criteria for cardiac response. If validated in larger international multi-site studies, these LS% cut-offs should be incorporated into new prognostic staging systems to more accurately assign prognosis at baseline and following therapy (algorithm proposed in **Figure 4.9 and 4.10** respectively).

Figure 4.9: Suggested baseline staging system for patients with cardiac AL amyloidosis incorporating longitudinal strain

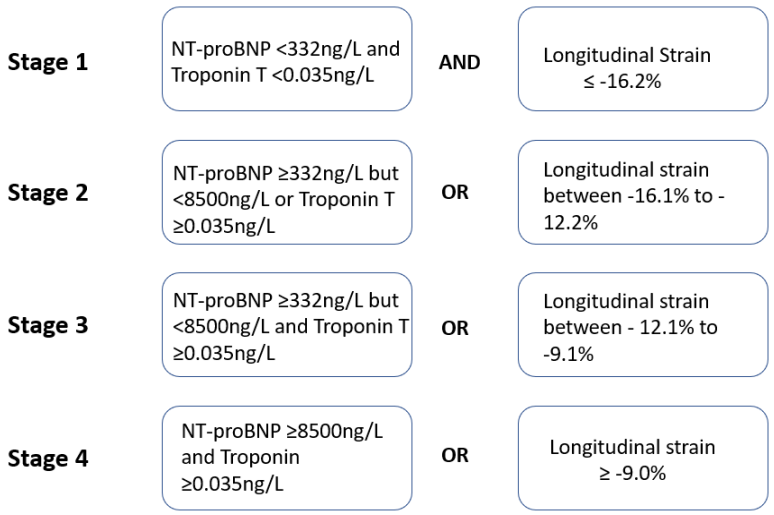
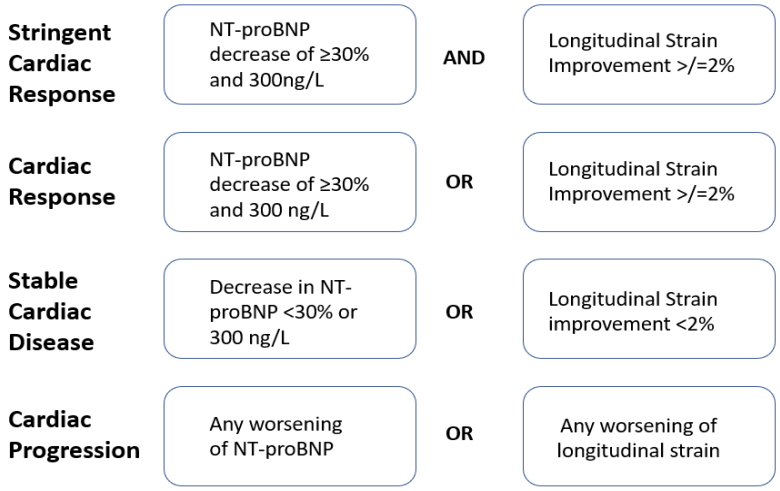


Figure 4.10: Suggested organ response criteria in patients with cardiac AL amyloidosis incorporating longitudinal strain



Since light chains are the drivers of disease in AL amyloidosis, we evaluated the impact of deep haematological responses using a previously defined threshold of dFLC <10mg/L (104). Longitudinal strain improved in patients achieving a complete haematological response and dFLC<10mg/L whilst this was not the case in patients achieving a CR without a dFLC<10mg/L. This is of great importance in guiding therapy and reinforces prior findings that a stringent dFLC response may represent a new goal of therapy in AL amyloidosis (104).

We acknowledge a number of limitations in this study inclusive of its single centre design and missing data at each time point reflective of patient loss to follow up. The study does was not designed to compare the prognostic impact of longitudinal strain with other echocardiographic parameters as these were not measured in all cases. Finally, strain analysis was performed in a 4-chamber view, which was

preferred to traditional global longitudinal strain to minimise the number of patients excluded given the challenges of obtaining good quality images in 2 or 3 chamber apical views. However, a good correlation between global longitudinal strain in the 3 apical views and four-chamber longitudinal strain has been demonstrated (196). In addition, LS% measurements may vary with the imaging software used to calculate it (197), which represents a potential drawback. The impact of these differences is likely small but needs to be further elucidated.

In conclusion, LS% at baseline predicts survival and is independent of the traditional biomarker-based scoring system used to define prognosis in cardiac AL amyloidosis. The LS% improves slowly following treatment in patients achieving a complete haematological response with a dFLC<10mg/L. An absolute improvement in LS% of -2.0% defines a group of patients with an improved survival than those with a biomarker-based response alone. These results support the use of LS% in baseline and serial assessments of these patients.

Chapter 5: The prognostic importance of the 6-minute walk test in AL Amyloidosis

This chapter is written in the context of my publication:

The prognostic importance of the 6-minute walk test in AL amyloidosis. OC Cohen, A Sathyanath, S Ravichandran, S Law, R Manwani, D Foard, S Sachchithananthan, S Mahmood, A Martinez-Naharro, M Fontana, CJ Whelan, PN Hawkins, HJ Lachmann, JD Gillmore, AD Wechalekar. Heart (2022); 108(20):1616-1622. Copyright permission obtained, as per copyright transfer agreement, for use in my thesis.

Introduction

Systemic AL amyloidosis, a condition characterised by misfolding of light-chain immunoglobulin and its deposition within organs, can often present with non-specific features such as fatigue and impaired functional capacity (198). Once diagnosed, assessment of fitness for treatment, response to treatment and stratification of long-term outcomes is based upon blood tests and imaging. The precise impact of functional capacity on survival is well recognised in heart failure from other causes (199) but remains difficult to capture in systemic amyloidosis. A standardised objective measure of function would provide additional data for use when assessing a patients fitness for chemotherapy, the impact of treatment and may guide prognosis. The 6MWT is one such measure, which is standardised, easy-to-administer and has been accepted by regulatory bodies as a valid clinical trial end-point in studies of cardiac failure and pulmonary hypertension (200).

The 6MWT involves walking across a flat surface for exactly six minutes at a self-selected pace to better reflect the level of exertion required for activities of daily living (164). A 6MWT distance of <300m predicts poor outcomes in patients with cardiac failure (201-204). Clinical trials of interventions in heart failure suggest that an increase of 30-50m may be considered clinically meaningful (205). A large study of nearly 400 patients with pulmonary hypertension concluded that the minimum important difference of the 6MWT in this subgroup was approximately 33m (206).

Data on utility of 6MWT in AL amyloidosis remain scarce. The Boston Medical Center amyloidosis group reported a small series of patients with and without cardiac involvement showing a reduction in the 6MWT in those with cardiac involvement correlating with worsening NYHA dyspnoea grade (207, 208). In AL amyloidosis, measurement of treatment response remains challenging and is based on surrogates such as cardiac biomarkers. A formal functional test to capture overall clinical improvement is required and the 6MWT has the potential to be used for this purpose.

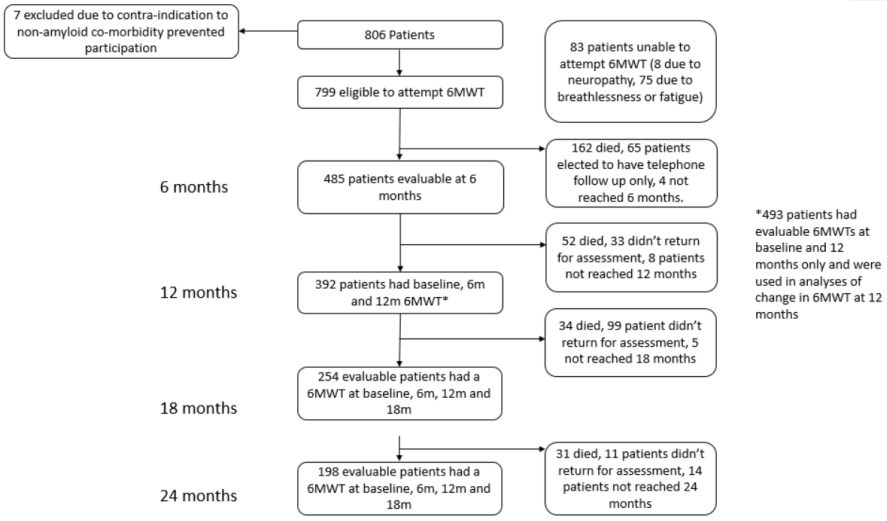
This study, the largest ever to evaluate the 6MWT in AL amyloidosis, hypothesises that the 6MWT is a sensitive prognostic marker to stratify patients at baseline in a treatment-naive population and following cytotoxic chemotherapy. We aim to validate the prognostic importance of this test.

Method

All patients from a prospective observational study of newly diagnosed treatment-naïve AL amyloidosis (ALchemy), seen at the UK NAC (October 2012 – August 2017) were included. The diagnosis of AL amyloidosis was confirmed by Congo red staining of a tissue biopsy whilst subtype was confirmed by

immunohistochemistry using specific antibodies or mass spectrometry. All patients had a detailed baseline assessment inclusive of both clonal markers and biochemical markers of organ function and echocardiography. This assessment was repeated at 6, 12, 18, 24, 36 and 48 month follow up. **Figure 5.1** shows a diagram of evaluable patients at each time point.

Figure 5.1: Flow diagram demonstration patients available for analysis at each timepoint



The 6-minute walk test was conducted in accordance with the American Thoracic Society guidelines (164). The result was used to calculate a percentage of the predicted value for age, sex, height and weight (165). Patients were excluded if they met criteria for any absolute or relative contraindication, namely resting heart rate >120 beats per minute, systolic blood pressure >180mmHg and/or diastolic blood pressure >100mmHg. A distance of <300m was initially chosen to represent a poor performance cut-off as previously reported as a useful prognostic marker of

subsequent cardiac death (201-204). There is limited published literature to define a clinically meaningful improvement in 6MWT. In the setting of cardiac rehabilitation post-myocardial infarction (209), chronic heart failure (210) and pulmonary hypertension (206), the minimum clinically important difference in 6MWT has been reported to be 25-33m. Consequently, we took the highest of these values and deemed 33m to represent a reasonable minimum value indicative of a meaningful improvement.

Haematological and organ responses were defined as per international consensus criteria (167, 172). Specifically, a haematological CR is defined by the absence of a detectable monoclonal protein with normalisation of the free light chain ratio. A VGPR represents a dFLC of <40mg/L whilst a PR represents a dFLC decrease of >50% from baseline. A cardiac response was defined by reduction in NT-proBNP (>30% and >300ng/L) assuming a baseline of ≥650ng/L or ≥2 class decrease in the NYHA class. Overall survival was defined as time from diagnosis to death from any cause.

Statistical analysis was performed using SPSS version 25 (IBM Corporation, Armonk, NY, USA). However, Stata (Stata 2021. State Statistical Software: Release 17. College Station, Texas USA) was used to perform the ROC analyses. Approval for analysis and publication was obtained from the National Health Service institutional review board; written consent was obtained from all patients in accordance with the Declaration of Helsinki. The Kaplan-Meier method was used to analyse survival outcomes. Multivariable modelling by Cox regression analysis was performed on factors found to significantly impact survival on univariate analysis. Pearson's correlation coefficient was used to calculate correlation. Non-parametric t-tests were used to compare continuous variables. In these cases, all p values were

2-sided with a significance level of <0.05. The Mann-Whitney U test was used to compare statistical variables in 2 groups and in such cases, a more stringent p value (<0.01) was used to determine significance to overcome potential issues associated with multiple testing. All walk test results are reported as median metres walked/median percentage predicted (e.g. 386m/74%).

Results

Eight hundred and seven patients were identified of which 7 patients were excluded on the basis of an initial contraindication (2 heart rate >120bpm, 5 blood pressure >180mmHg/>100mmHg). One patient was excluded due to immobility secondary to Charcot-Marie-Tooth disease. Baseline characteristics are included within **Table 5.1**. Eighty-three patients (10.4%) were unable to attempt the test secondary to symptoms associated with their amyloidosis, which was breathlessness in 75 cases. Of 51 (6.4%) patients with peripheral neuropathy and 48 (6.0%) patients with autonomic neuropathy, 4 patients in each category were unable to attempt the test as a result of these symptoms.

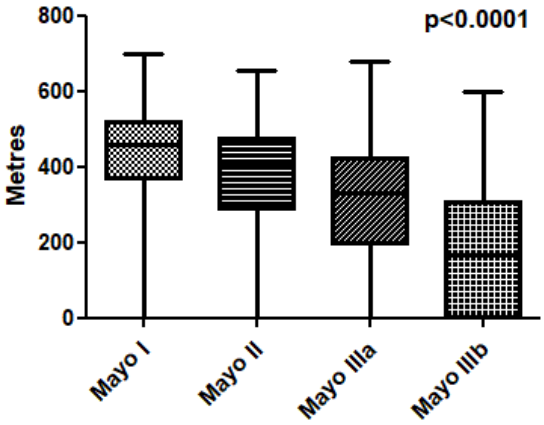
Table 5.1: Baseline Patient Characteristics by distance achieved on baseline 6-minute walk test

	Median (range) or n (%)			
	Overall (n=799)	≤350m (n=377)	>350m (n=422)	P value
Age (years)	69 (32-92)	66.5 (34-89)	69 (32-92)	0.14
Males / Females	465 (58.2) / 334 (41.8)	199 (50.1) / 178 (49.9)	266 (63.0) / 156 (37.0)	0.20
NYHA Class				0.25
1	207 (25.9)	60 (15.9)	147 (34.8)	
2	394 (49.3)	192 (50.9)	202 (47.9)	
3	82 (10.3)	73 (19.4)	9 (2.1)	
4	4 (0.5)	4 (1,1)	0 (0)	
Unrecorded	112 (14.0)	48 (12.7)	64 (15.2)	
ECOG				0.89
0	192 (24.0)	22 (5.8)	170 (40.3)	
1	314 (39.3)	118 (31.3)	196 (46.4)	
2	221 (27.7)	183 (48.5)	38 (9.0)	
3	36 (4.5)	35 (9.3)	1 (0.2)	
Unrecorded	36 (4.5)	19 (5.0)	18 (4.3)	
Kappa / Lambda	168 (21.0) / 631 (79.0)	90 (23.9) / 295 (76.1)	78 (18.5) / 336 (81.5)	0.97
Median dFLC, mg/L	168 (0-15898)	242 (0-4029)	133 (2-15898)	0.46
Cardiac Involvement	564 (70.6)	273 (72.4)	291 (69.0)	0.03
NT-proBNP, ng/L	2174 (25-93776)	4178 (110-93776)	1136 (25-51237)	0.01
LV wall thickness (mm)	13 (6-22)	13 (9-21)	12 (6-20)	0.001
Mayo Stage				0.001
I	125 (15.6)	28 (7.4)	97 (23.0)	
II	261 (32.7)	97 (25.7)	164 (38.9)	
IIIa	286 (35.8)	160 (42.4)	126 (29.9)	
IIIb	101 (12.6)	83 (22.0)	18 (4.3)	
Unrecorded	26 (3.3)	9 (2.4)	17 (4.0)	
Renal Involvement	552 (69.1)	232 (61.5)	320 (75.8)	
Serum creatinine, µmol/L	98 (26-979)	101 (26-610)	95 (30-979)	0.15
GFR, ml/min	64 (<15->90)	59 (<15->90)	68 (<15->90)	0.15
Proteinuria, g/24h	3.3 (0-36)	2.3 (0-16)	4.3 (0-36)	0.68
Albumin, g/L	34 (13-53)	35 (17-51)	53 (13-53)	0.60
Liver involvement	113 (14.1)	53 (14.1)	60 (14.2)	0.87
No. organs involved	2 (1-5)	2 (1-5)	2 (1-5)	0.93

Abbreviations: NYHA: New York Heart Association Classification of Heart Failure; ECOG: Eastern Cooperative Oncology Group Performance Status; dFLC: difference between involved and uninvolved free light chains; NT-proBNP: N-terminal pro hormone brain natriuretic peptide; LV: Left ventricle; eGFR: estimated glomerular filtration rate.

The median 6MWT of the cohort was 362m (0-700m). Five-hundred and sixty four (70.6%) patients had cardiac involvement. These patients walked less far at baseline (cardiac vs. non-cardiac: 337.0m/64% vs. 421.0m/84% [p<0.0001]). The 6MWT shortened with increasing Mayo stage (Stage I – 458.0m/91.0%, Stage II – 404.0m/80.0%, Stage IIIa – 331.0m/65.0%, Stage IIIb – 168.0m/34.0% [p<0.0001]) (**Figure 5.2**). Finally, a shorter baseline 6MWT correlated significantly with other prognostic measures of functional status such as Eastern Cooperative Oncology Group performance status (-0.629, p=0.01) and NYHA stage (-0.397, p=0.01).

Figure 5.2: Baseline 6-minute walk distance by Mayo Stage



Patients were followed up for a median of 32.0 (range: 1.0 - 90.0) months. Median OS from diagnosis was 70.0 (95% CI: 56.8 - 83.2) months (**Figure 5.3**) overall but 32.0 (95% CI: 23.1 - 40.9) months in patients with cardiac involvement. Using the traditional 6MWT cut-off of 300m, median OS of patients achieving this distance was not reached (vs. 25.0 [95% CI: 18.1 - 31.9] months if <300m

(p<0.0001). However, given that this cut-point was not specifically designed for patients with amyloidosis, we used a ROC analysis to identify the optimal point to predict survival within our cohort. Given the many factors that can impact the 6MWT, sensitivity and specificity were expectedly low across most time points. A value of 350m (sensitivity 33.1%; specificity 30.4%) was the most discriminatory. The OS of patients achieving ≥350m at baseline was significantly longer than those achieving <350m. This effect was maintained when patients with and without cardiac involvement were analysed separately (p<0.0001 in both cases). Patients unable to attempt the test had an especially poor OS of 5.0 (95% CI: 2.8-7.2 months) (**Figure 5.4**). In patients with Mayo IIIb biomarkers, the 6MWT remained prognostic (≥350m: 59 [95% CI: 4.2 – 149.4] months vs. <350m: 4 (95% CI: 1.3 – 6.7) months vs no attempt: 1 (95% CI: 0.3 – 1.7) months [p<0.0001]) (**Figure 5.5**).

Figure 5.3: Overall survival of all patients

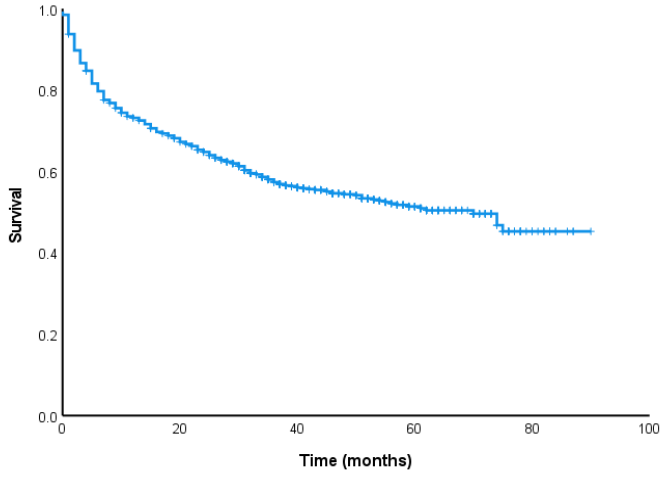


Figure 5.4: Overall survival of patients by baseline 6-minute walk distance

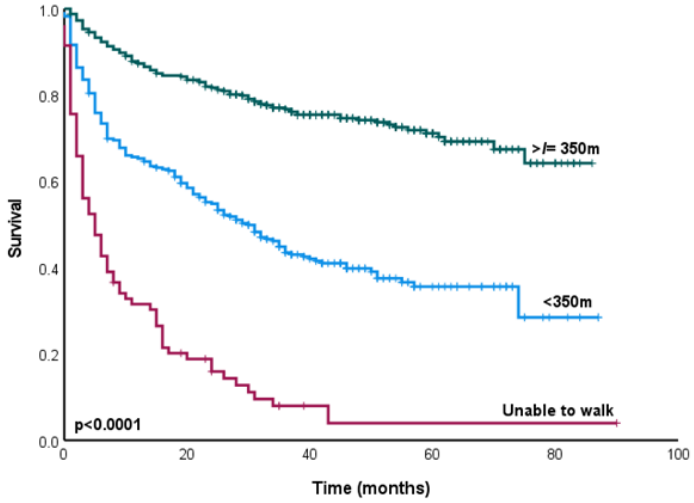
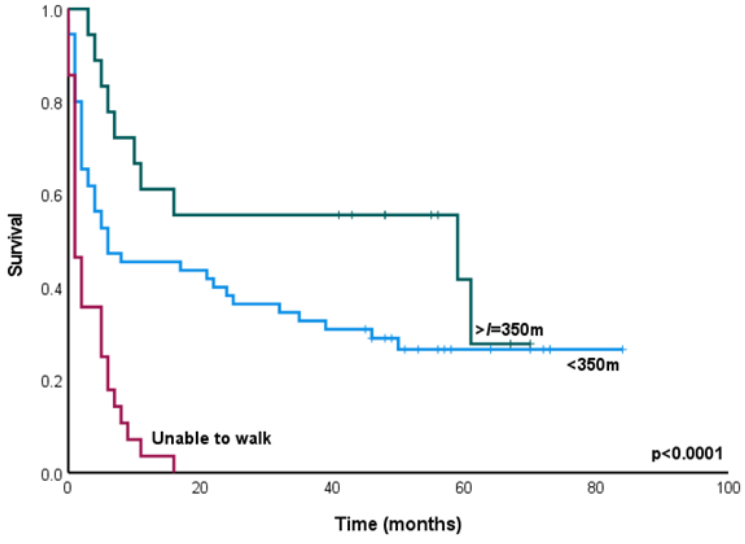


Figure 5.5: Overall survival of patients by baseline 6-minute walk distance in patients with Mayo IIIB cardiac biomarkers at baseline



On multivariable analysis, a baseline 6MWT of ≥350m independently predicted better outcomes in a model incorporating Mayo staging (**Table 5.2A**).

Table 5.2: Multivariable models incorporating:

A) Mayo staging and 6-minute walk test ≥ 350m at baseline

	Hazard ratio	95% confidence interval	P value
Mayo I (reference)			
Mayo II	2.22	1.35-3.64	0.002
Mayo IIIa	3.83	2.37-6.21	<0.0001
Mayo IIIb	6.04	3.61-10.10	<0.0001
6MWT > 350m	2.74	2.16-3.47	<0.0001

B) Haematological response and change in 6-minute walk test (reduction of ≥33m) at 12 months in patients with cardiac AL amyloidosis

	Hazard ratio	95% confidence interval	P value
CR (reference)			0.029
VGPR	2.02	1.08-3.80	<0.0001
PR	3.51	1.83-6.73	<0.0001
NR	5.61	2.88-10.92	
∆6MWT ≥ 33m	1.61	1.01-2.59	0.047

Abbreviations: CR: complete response; VGPR: very good partial response; PR: partial response; NR: no response; 6MWT: 6-minute walk test.

C) Haematological response and change in 6-minute walk test (reduction of ≥44m) at 12 months in patients with cardiac AL amyloidosis

	Hazard ratio	95% confidence interval	P value
CR (reference)			
VGPR	1.49	0.75-2.96	0.26
PR	2.90	1.41-5.96	0.004
NR	4.72	2.07-10.80	<0.0001
∆6MWT ≥ 44m	1.76	1.00-3.11	0.043

Abbreviations: CR: complete response; VGPR: very good partial response; PR: partial response; NR: no response; 6MWT: 6-minute walk test.

Next, the 6MWD was evaluated according to haematological response. Three-hundred and fifty eight patients had evaluable data at each of the baseline, 6 and 12 month timepoints. Patients who did not have data available at each time point were excluded to ensure a comparable analysis. In evaluable patients, haematological responses were: CR – 91 (25.4%), VGPR – 142 (39.7%), PR – 78 (21.8%) and NR – 47 (13.1%) [p=0.19 for baseline 6MWT between groups]. The 6MWD decreased at 6 months across all categories but most markedly in those patients with a poorer haematological response – at 6 months: CR – 17.0m (p=0.004), VGPR – 23.5m (p=0.003), PR – 48.0m (p=0.001) and NR – 77.0m (p=0.001). At 12 months, patients in a CR walked significantly further than those in a VGPR (437.0m/85.0% vs. 395.5m/76.5%, p=0.009). Uniquely, patients achieving a CR showed improvement in 6MWD between 6 and 12 months (414m/79.0% to 437m/85.0%, p=0.001). The 6MWD did not improve further at the 18 month and 24 month timepoints in patients achieving a CR (p=0.23 and p=0.11 respectively). Patients achieving a cardiac organ response (n=125) at 12 months also improved their 6MWD from baseline (414m/79.0% to 437m/85.0%, p=0.001).

Significant differences between Mayo stage I, II and II persisted at 12 months (Stage I: 435.0m/85.0%; Stage II: 395.0m/79.0%, Stage III: 345.0m/67.0%, p<0.0001). Thirty-one patients with Mayo IIIb disease returned for assessment at 12m. These patients had a median baseline 6MWT of 300.0m/56.0% (n=31) in comparison to just 92.0m/18.5% in those who did not return at 12 months (n=70, 64/70 [91.4%] had died prior to 12 months; 6 lost to follow up) (p<0.0001).

An improvement of ≥33m was previously considered to be the minimum clinically meaningful increase in 6MWD as described above (206) whilst ≥44m was identified as optimal within this study population (sensitivity 61.3%; specificity

69.1%). In total, 28.2% and 25.8% improved by 33m and 44m respectively. This increased to 36.6%/31.5% and 47.2%/43.2% in patients improving their walk test by ≥33m/≥44m and who had achieved a complete haematological and cardiac organ response. Using either cut-point, patients with cardiac involvement who improved their 6MWD had a greater overall survival (**Figure 5.6/5.7 respectively**). This effect was lost in patients without cardiac involvement (p=0.40 and p=0.46 respectively). On multivariable analysis, an improvement in 6MWT of ≥33m/≥44m was independent of haematological response in predicting better survival in patients with cardiac AL amyloidosis (**Table 5.2 B/C**). In a landmark analysis of patients with Mayo stage III disease alive at 12 months those patients who improved by ≥33m at 12 months lived longer (OS NR vs. 70.0 [51.4-88.6] months, p<0.0001), which yet again was replicated using the 44m cut-off.

Figure 5.6: Overall survival by improvement in 6-minute walk distance of ≥33m at 12 months in patients with cardiac AL amyloidosis

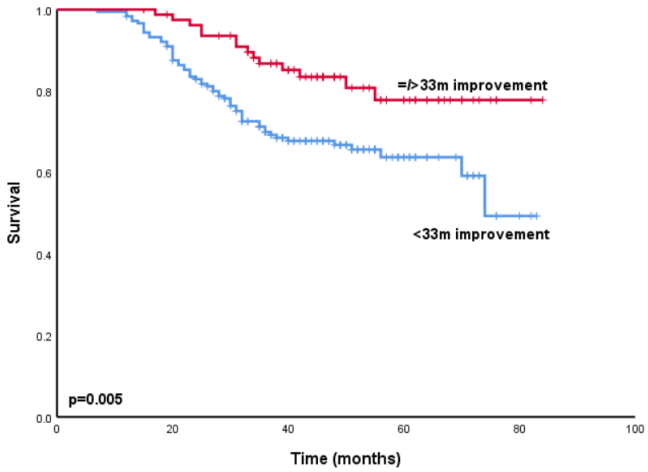
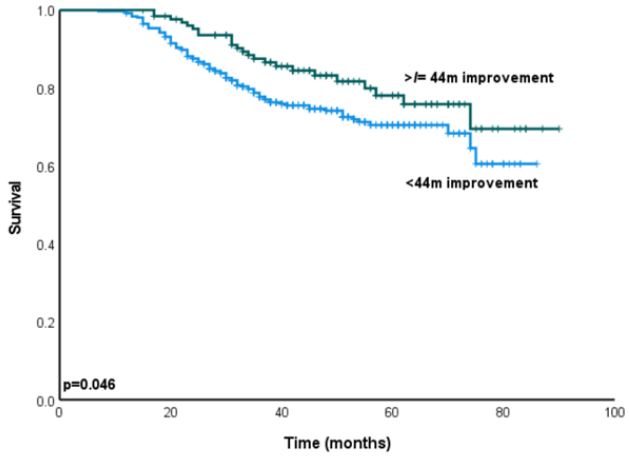


Figure 5.7: Overall survival by improvement in 6-minute walk distance of ≥44m at 12 months in patients with cardiac AL amyloidosis



Discussion

This study demonstrates the prognostic value of the 6MWT in a large cohort of uniformly treated patients with systemic AL amyloidosis. Baseline 6MWT independently predicts survival and a distance of <350m identifies patients with a shorter prognosis. Prognosis is particularly poor in patients unable to attempt the test. At 12 months, the 6MWD only improves in patients who achieve a complete haematological or cardiac organ response. An improvement in 6MWD at 12 months is predictive of survival.

In a range of diseases, the role of functional assessments, such as 6-minute walk testing, is well established. The test has been shown to be prognostic in patients with left-ventricular dysfunction (211) and applied to other conditions inclusive of pulmonary hypertension (206), respiratory disease (212) and renal failure (213). In transthyretin-type amyloidosis, 6MWD was used as a trial end-point to demonstrate the slower rate in functional decline of patients receiving Tafamidis

compared to placebo (28). In systemic AL amyloidosis, the prognostic value of the 6MWT has been demonstrated (208) but no specific cut-offs have been published based upon a cohort of patients with this disease. A walk test of <300m defines patients in a poor prognostic category in cardiovascular disease (201-204) but <350m was deemed optimal in the evaluation of patients with AL amyloidosis. Following further validation, this cut-point could be applicable as an inclusion/exclusion criterion in a clinical trial setting.

The Mayo criteria still form the basis of disease prognostication in systemic AL amyloidosis. This study demonstrates that patients with a higher Mayo stage achieve lower 6MWDs. Patients with Mayo Stage IIIb disease have an especially poor prognosis of just 3-6 months (22). However, if such patients have a baseline 6MWT ≥350m (19.0% of the stage IIIb patients) the median OS is 59.0 months (vs. 4.0 months if 6MWT <350m). Patients unable to attempt the 6MWT at baseline have a poor OS of just 5 months irrespective of Mayo stage. These findings suggest the utility of 6MWT in improving the risk stratification of patients at baseline although again, this requires validation in larger clinical trials with higher patient numbers.

In systemic AL amyloidosis, a deeper haematological response is associated with longer survival (198). In patients who fail to achieve at least a VGPR to first-line therapy, further treatment is typically advocated (214). It was previously reported that the 6-minute walk distance was stable or had improved in patients attaining at least a VGPR at 12 months (207). However, this study showed that only patients in a complete haematological response improved their walk test between 6 and 12 months. Furthermore, patients in a CR walked significantly further than those in a VGPR at 12 months. This follows a dip in 6MWD at 6 months, which is likely associated with worsening of amyloid deposition in organs after diagnosis but prior to

the attainment of a deep haematological response and the impact of chemotherapy-induced toxicity (fatigue, neuropathy). This worsening of 6MWD was least marked in patients achieving a deep haematological response. This study also demonstrated the impact of a cardiac organ response on 6MWD, which corroborates previous data from Decker *et al* who demonstrated that patients achieving a cardiac response improve their median 6MWT (215).

A defined "clinically meaningful" improvement in 6MWD has not been defined in AL amyloidosis but values in the range of 25-33m have been postulated previously to apply in other conditions (206, 209, 210, 212). We used the largest of these values (33m) as a minimum value representative of clinically meaningful improvement but also took the higher value of 44m based on a ROC analysis of our subjects. Of note, the sensitivity and specificity chosen was only marginally higher than other similar values, which is the reason why both cut-points were analysed and reported. Both cut-offs were associated with improved survival. Both cut-offs provided incremental information over and above haematological response, a factor known to strongly predict survival in this setting. The true optimal value to take forward as a potential end-point in clinical trials in systemic AL amyloidosis remains unclear and requires further exploration.

This study is limited by its single centre design and loss of patients to follow up at each time point. However, each analysis only incorporated patients with data available at each relevant timepoint to ensure that the analysis was comparable. This study does not account for the potential learning effect from repeated attempts at the 6MWT over time but prior studies have suggested that this learning effect does not persist at 6 months (216). Furthermore, many patients with AL amyloidosis

would be unable to undergo the 6MWT on two occasions at the same consultation so further exploration of this learning effect would be practically difficult to implement.

Chapter 6: The role of serial health-related quality of life assessment in AL amyloidosis

This chapter is written in the context of my publication:

Linking changes in quality of life to haematologic response and survival in systemic amyloidosis. Cohen OC, Rendas-Baum R, MxCausland K, Foard D, Manwani R, Ravichandran S, Lachmann HJ, Mahmood S, Wisniowski B, Hawkins PN, Gillmore JD, Hsu K, Rebello S, Wechalekar AD. British Journal of Haematology (2023). 201(3):422-431. Copyright permission obtained, from Wiley, as per copyright transfer agreement, for use in my thesis.

Introduction

Systemic AL amyloidosis is characterised by, often devastating, organ dysfunction occurring secondary to the deposition of a light-chain immunoglobulin originating from a clonal cell population in bone marrow (185). It is increasingly recognised that assessment of organ function by blood-based biomarkers and imaging techniques does not reflect the true burden of the disease in its entirety. The use of a multi-dimensional measure of the impact of a particular disease upon both physical function and mental health is paramount given that quality of life is reported to be as important to patients as survival itself (217). There is increasing recognition amongst regulatory bodies, such as the Food and Drug Administration, of the value of patient-reported outcomes in supporting labelling claims for approval of

new therapies (218) and yet capturing global improvement in a patient's condition has yet to be studied thoroughly in this multi-system disease.

A patient's own reflection of their treatment experience can capture key information relating to tolerability. Poor tolerance of treatment negatively impacts health-related quality of life whilst effective treatments lead to improvement in HRQL over time (219-221). Despite reports of HRQL scores following initiation of treatment, data examining the impact of depth of haematological response, a key determinant of patient outcomes, on HRQL is lacking.

This study evaluates change in HRQL in a cohort of newly diagnosed, bortezomib-treated patients who were participating in a large real-world study of AL amyloidosis (ALchemy) and explores the association of baseline HRQL and change in HRQL after therapy with haematological response depth. Prior studies demonstrate that patients achieving deep haematological responses had durable remissions with 78% of patients alive and 71% remaining progression-free at 5 years (104) and yet, despite encouraging outcomes, the impact of HRQL on patients with AL amyloidosis, remains underreported.

Methods

Newly-diagnosed patients with AL amyloidosis, presenting to the UK NAC for evaluation and commencing on bortezomib-based therapy were prospectively enrolled on the (ALchemy) study. Baseline data inclusive of HRQL via the SF36v2 as well as markers of organ function was collected at baseline, 3, 12 and 24 months. Patients were asked to send monthly blood samples to the NAC to allow haematological response monitoring to occur on a monthly basis. Patients were also

evaluated with a baseline echocardiogram and ^{123}I-SAP scintigraphy was performed. Patients who did not complete the SF-36v2 at baseline were excluded.

Haematological and organ responses were defined as per consensus guidelines (167, 172). HRQL was measured using the SF-36v2, a generic survey that results in scores for eight dimensions of functional health and well-being: Physical Functioning (PF), Role-Physical (RP; role limitations due to physical problems), Bodily Pain (BP), General Health Perceptions (GH), Vitality (VT), Social Functioning (SF), Role-Emotional (RE; role limitations due to emotional problems), and Mental Health (MH) (222). In addition, two summary scores (Physical Component Summary [PCS] and Mental Component Summary [MCS]) are calculated through a linear combination of the eight domain scores. All SF-36v2 scores are designed to have a mean of 50 and a standard deviation of 10 based on the U.S. general population. Thus, scores above and below 50 are above and below the U.S. general population average, respectively, with higher values on all SF-36v2 scores implying better HRQL. Interpretation of group differences and individual changes in SF-36v2 scores can also be made using thresholds that have been developed for this purpose (222). The current study uses these values to better interpret findings related to HRQL scores.

Mean change from baseline for every SF-36v2 score was calculated to evaluate changes in the HRQL of patients experiencing different levels of haematological response. The primary analyses relied on all available cases at two timepoints where haematological response and HRQL were both assessed: six and 12 months after treatment initiation**.** A secondary set of analyses relied on the subset of patients who were alive at least 24 months after baseline. HRQL change was also evaluated among patients with and without cardiac response, as cardiac involvement is highly prognostic

of survival. Statistical analyses were conducted using SAS/STAT software, Version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA) and R 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria, JM package, version 1.4-8).

Results

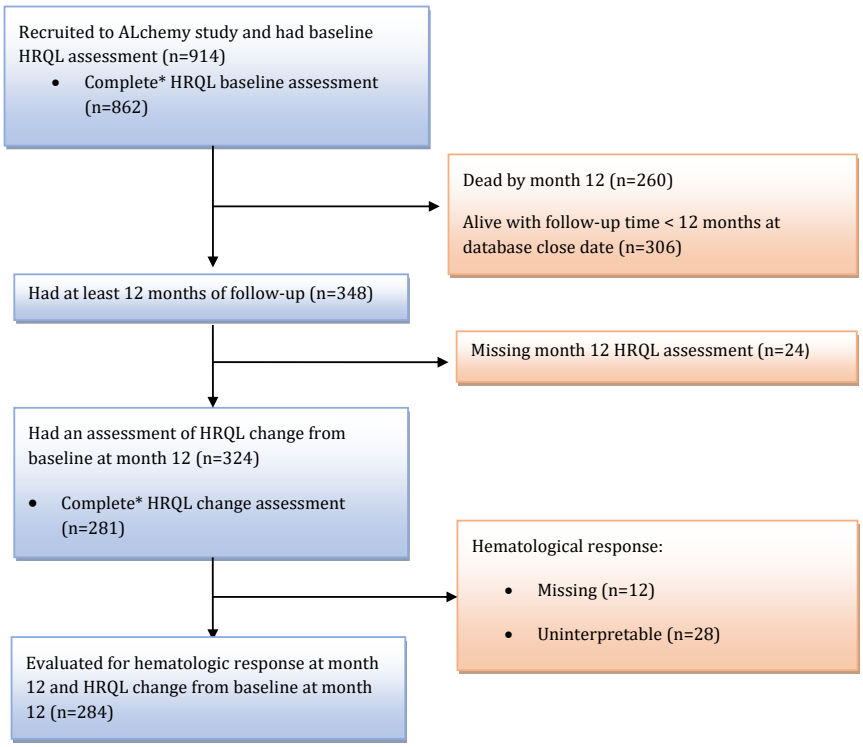
Patients

Data from 914 patients were used in the analyses (**Table 6.1**). Approximately 40% of patients were female, and median age was 69 years (range, 39-90). Cardiac involvement was present in 63% (n=578) of patients, 69% (n=628) had renal involvement, and 38% (n=347) had both cardiac and renal involvement. Patients were classed as: Mayo Stage I (n=153; 17%), Mayo Stage II (n=309; 34%), Mayo Stage IIIa (n=337; 37%), and Mayo Stage IIIb (n=106; 12%). Median NT-proBNP and dFLC were 2,097 ng/L (range, 0-70,000) and 180 mg/L (range, 0-15,898), respectively. A total of 328 patients died during the study period, with 79.3% (n=260) of these dying within 12 months of initiating treatment (**Figure 6.1**). From amongst the remaining 654 patients, a total of 348 were observed for 12 months or longer following treatment initiation. At the 12-month study visit, a total of 284 patients were assessed for both HRQL and haematological response (**Figure 6.1**). The percentage of patients with CR or VGPR at 12 months was 63% (n=102 and n=78, respectively; **Table 6.2**). Organ response, evaluated 12 months following treatment initiation, indicated that among the 181 patients with cardiac involvement at baseline and a HRQL change score at 12 months, 152 had evaluable data (uninterpretable: n=20; missing: n=9) and 71 (39.2%, on an intent to treat basis) had a cardiac response.

Table 6.1: Baseline Patient Characteristics at time of completing initial SR-36v2

	N(%)/Median(range)
Age, median (range)	69 (39-90)
Male, N (%)	560 (61.3)
ECOG class	
0	220 (24.1)
1	321 (35.1)
2	268 (29.3)
3	42 (4.6)
Not recorded	63 (6.9)
dFLC	
<20mg/L	65 (7.1)
20-50mg/L	56 (6.1)
>50mg/L	737 (80.6)
Not recorded	56 (6.1)
Mayo Stage at Presentation	
1	153 (16.7)
2	309 (33.8)
3A	337 (36.9)
3B	106 (11.6)
Not recorded	9 (1.0)
Organ Involvement	
Cardiac	578 (63.2)
Renal	628 (68.7)
Liver	119 (13.0)
Soft Tissue	146 (16.0)
Peripheral Nerve	85 (9.3)
Autonomic Nerve	86 (9.4)
Gastrointestinal	44 (4.8)
No. of organs	2 (1-5)
Baseline Organ Function	
Creatinine, µmol/L	96 (27-1077)
Proteinuria, g per 24h	3 (0-33)
NT-proBNP, ng/L, median (range)	2097 (0-70000)
CKD Stage	
1	190 (20.8)
2	325 (35.6)
3	246 (26.9)
4	107 (11.7)
5	40 (4.4)
Not recorded	6 (0.7)
Systolic blood pressure	118 (0-190)
6-minute walk test	350 (0-708)

Figure 6.1: Flow diagram depicting patients evaluable at each time point



**Complete assessment indicates that responses to all SF-36v2 questions were provided and were within valid range, allowing for calculation of all 10 SF-36v2 scores.*

Table 6.2: Mean Change from Baseline to Month 12 in SF-36v2 Scores by Haematological and Cardiac Response

SF-36v2 Score *†	Haematological Response					Cardiac	
	Complete Response (n=102)	Very Good Partial Response (n=78)	Partial Response (n=67)	No Response (n=37)		Response (n=71)	No Response (n=81)
PF	**2.07**	**-0.88**	-3.39	-5.31±		**4.15**	-1.38
RP	**2.93**	**1.76**	-2.55	-3.85		**5.49**±	0.49
BP	**2.69**	-0.23	-1.39	-1.67		2.02	0.28
GH	**0.30**	**-3.03**	-3.99	-5.89		**0.77**	-2.37
VT	**1.39**	-1.11	-1.08	-2.35		1.76	1.23
SF	**4.36**	1.39	-4.41	-1.53		**5.93**	1.35
RE	3.50	2.90	-1.07	0.40		4.15	2.04
MH	**5.39**	2.12	1.48	-0.29		4.90	3.61
PCS	**0.86**	**-1.81**	-4.23±	-4.89±		**2.35**	-1.93
MCS	**4.55**	2.66	0.17	0.66		4.29	3.67

Note: Mean changes in SF-36v2 scores across haematological response levels were based on 284 patients; mean changes in SF-36v2 scores across levels of cardiac response were based on 152 patients; values shown in bold font indicate a difference from the "No Response" group ≥ (in absolute value) the smallest value indicating a meaningful between-group difference.

* Smallest values indicating a meaningful individual-level change: PF, 4.3; RP, 4.0; BP, 5.5; GH, 7.0; VT, 6.7; SF, 6.2; RE, 4.6; MH, 6.7; PCS, 3.8; MCS, 4.6

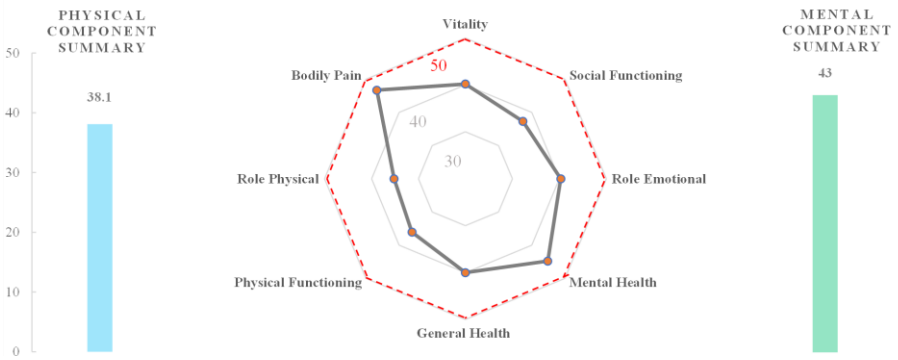
† Smallest values indicating a meaningful group-level difference: PF, 3; RP, 3; BP, 3; GH, 2; VT, 2; SF, 3; RE, 4; MH, 3; PCS, 2; MCS, 3

± Indicates an average within-person change ≥ the smallest meaningful individual-level change.

Quality of Life Scores

SF-36v2 scores showed large decrements in HRQL at baseline with mean scores between 35.2 (RP) and 46.7 (BP) across the 8 domains (**Figure 6.2**).

Figure 6.2: Health Related Quality of Life at Baseline: SF-26v2 scores

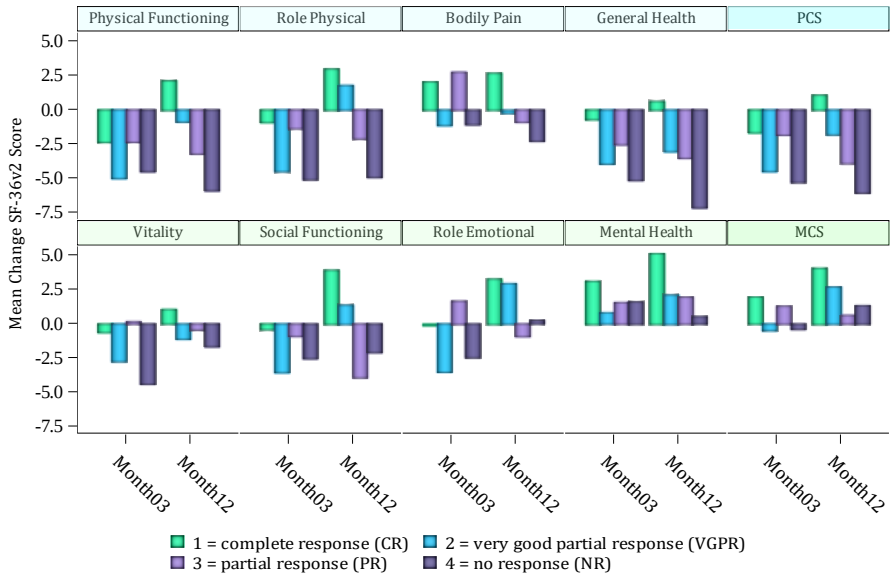


At 3 months, HRQL scores declined following commencement of chemotherapy. At this timepoint, PCS declined by an average of -3.6 points, which exceeds the minimum meaningful within-person change for this score (3.4 points(222)). The consistent absolute decline in scores at 3 months in 5/8 categories (PF, RP, GH, VT and SF; **Figure 6.3**) as well as PCS occurred irrespective of haematological response. By 12 months, PCS had declined by an average of -2 points relate to baseline. Conversely, the change in MCS, BP and MH was minimal. In patients achieving a CR at 12 months, there was a mean improvement in three SF-36v2 domains (**Figure 6.3**): PF (3.3; n=46), RP (5.5; n=45) and SF (8.0; n=44). There was not a significant improvement in the other domains nor did patients in <CR achieve a meaningful mean change in HRQL domains. Patients achieving a PR

or NR demonstrated a decline across nearly all domains. These trends persisted when only patients surviving >24 months were included within the analysis.

Cardiac response at 12 months was associated with improvement in HRQL across all SF-36v2 scores although differences in VT, MH, BP and MCS did not reach clinically meaningful levels (<2 in all cases). Concordant with haematological response, the largest mean gains in those achieving a cardiac response were seen in PF (4.2), RP (5.5) and SF (5.9). Differences between those achieving a cardiac response and those not achieving a cardiac response were also largest amongst these domains.

Figure 6.3: Mean change from baseline in SF-36v2 scores by haematological response



Discussion

This study evaluated whether meaningful changes in HRQL are associated with deeper haematological responses among a large sample of patients with AL amyloidosis, treated with upfront bortezomib-based regimens. At baseline, patients reported significant impairment of HRQL levels, in line with previously published studies (220).

Although reports exist that evaluate HRQL in patients with AL amyloidosis pre- and post-treatment, none have evaluated a link between depth of haematological response and HRQL. Our results indicate that patients with a CR report meaningful HRQL improvement after 1 year of treatment, reflected primarily in functioning domains (physical, role and social functioning). Patients in lesser haematological responses did not, on average, report meaningful QoL gains and in fact, patients in a <PR report a HRQL decline. Patients achieving a cardiac response report similar profile of responses to those in a CR with a focus on the same 3 functional domains. For the first time, we can demonstrate that deep haematological and organ responses to treatment translate to a patient 'feeling better', which is not only a key goal of therapy but also an evaluable aspect of the effectiveness of therapy by global regulators. However, improvement does take time – there was no improvement at 3 months, at which point patients would still be undergoing chemotherapy and would generally be yet to achieve an organ response.

The marked decline in HRQL at the 3-month time point reflects results from the Medical college of Wisconsin and Mayo Clinic study, which demonstrated worsening of multiple domains in the first 3 months of therapy (221). Other studies have also recognised that patients may show early symptomatic and biomarker deterioration prior to improvement, particularly in those with advanced disease (223).

In the Wisconsin/Mayo study, both physical and mental summary scores as well as those for physical and fatigue domains, showed a mean worsening in the first 3 months after treatment was initiated. Nevertheless, after 12 months, scores were improved compared to the 3-month timepoint, suggesting overall trajectories similar to those observed here. Despite this similarity, the Wisconsin/Mayo cohort study provided limited information on the association of haematological response and HRQL to patients with and without VGPR (n=22 and n=15 respectively). Our results greatly expand on this and evaluate all haematological responses across more time points and in greater numbers. The striking fact that only patients achieving at least a CR achieve true improvement adds weight to the notion that CR should be the minimum aim of treatment in patients with AL amyloidosis, especially in those with cardiac involvement as outlined in Chapter 4.

It is well established that organ responses in amyloidosis are linked to depth of haematological response. The current analysis demonstrates that patients' HRQL is concordant with this data. This also raises key questions relating to the goal of therapy in AL amyloidosis. This also supports recent publications, which show better outcomes and longer times to next treatment in patients achieving a CR (104). With respect to differentiation across the various HRQL domains, our results indicated that, while most SF-36v2 domains were impacted by treatment, those related to physical functioning, namely PF, RP, and SF, ranked more highly in magnitude of change and were also more clearly linked to depth of hematologic response, which is in agreement with previous studies (224).

In conclusion, our study shows that HRQL assessment is an essential element in characterising treatment response. It should be noted that the assessment instrument (i.e. what constituted meaningful changes in HRQL) was

developed with the general population in mind. Future studies should incorporate measurement of HRQL into the evaluation of treatment benefit, using instruments that specifically target concepts and symptoms that more fully reflect those most important to patients with AL amyloidosis.

Results Section 2: Novel therapies in systemic AL amyloidosis

Chapter 7: The use of ixazomib, lenalidomide and dexamethasone in systemic AL amyloidosis

This chapter is written in the context of my publication:

Use of ixazomib, lenalidomide and dexamethasone in patients with relapsed amyloid light-chain amyloidosis. Cohen OC, Sharpley F, Gillmore JD, Lachmann HJ, Sachchithanantham S, Mahmood S, Fontana M, Whelan CJ, Martinez-Naharro A, Kyriakou C, Rabin R, Popat R, Yong K, Cheesman S, Shah R, Hawkins PN, Wechalekar AD. British Journal of Haematology (2020). 189; 643-649. Copyright permission obtained, from Wiley, as per copyright transfer agreement for use in my thesis

Introduction

Systemic AL amyloidosis is characterised by the misfolding of light-chain immunoglobulin, produced by a plasma (or B cell) clone in bone marrow, which deposit in organs, leading to progressive dysfunction (225). Outcomes in AL amyloidosis are improving such that, over the last 40 years, the 4-year overall survival has doubled from 21% (1977-1986) to 42% (2003-2006) (226). This is likely attributable to the development of novel agents for use in AL amyloidosis and improved patient selection for autologous transplantation (99, 227).

Therapy aims to suppress the amyloidogenic light chain to attain a deep haematological response without incurring additional treatment-related organ toxicity over and above that caused by amyloid deposition (89). Bortezomib-based

regimens remain the mainstay of upfront treatment, with 60% of patients achieving a good haematological response (103), due to enhanced susceptibility of plasma cells in AL amyloidosis to proteasome inhibitor led killing (228). However, the majority of patients will relapse leading to an increased need to develop novel agents. In the relapse setting, lenalidomide-dexamethasone is commonly utilised (229) either alone or in combination with cyclophosphamide (21) or melphalan (109) leading to haematological response rates of 60% and 58% respectively. Response to lenalidomide is limited by tolerability.

Ixazomib is an oral proteasome inhibitor, which has been assessed in a phase 1/2 study in relapsed/refractory AL amyloidosis demonstrating a 52% haematological response (119). A phase III trial of ixazomib-dexamethasone vs. physician's choice closed early due to failure to meet primary endpoints but despite that reported a 53% haematological response rate and time to event data, which favoured the ixazomib-dexamethasone arm (120, 127). Furthermore, a phase II trial examining the combination of ixazomib-cyclophosphamide-dexamethasone led to an overall haematological response of 57% in thirty-five patients (230). The combination of ixazomib-lenalidomide-dexamethasone is established in multiple myeloma and demonstrates significantly longer PFS than lenalidomide-dexamethasone alone (231). Here, the real-world use of IRd in patients with relapsed systemic AL amyloidosis is reported for the first time.

Method

All patients with systemic AL amyloidosis, treated with ixazomib-lenalidomide-dexamethasone were identified from the database at the UK NAC (2016-2019). In all

cases, the diagnosis of amyloidosis was confirmed by Congo red staining of a tissue biopsy with demonstration of characteristic birefringence under cross-polarised light. The amyloid subtype was confirmed by immunohistochemistry with specific antibodies, or by mass spectrometry (232). A comprehensive baseline assessment was performed in all patients inclusive of clonal markers and blood markers of organ function. Patients also underwent echocardiography at baseline. Organ involvement and both haematological and organ response were determined as per international consensus criteria (167, 172). Responses were assessed at 3 months and best response achieved whilst on therapy. Progression was defined as haematological progression or death. Primary outcomes were haematological response and overall survival. Overall survival was calculated from commencement of IRd until death from any cause whilst progression-free survival was a secondary outcome, calculated from commencement of IRd to haematological progression or death from any cause. All treatment and survival outcomes are reported on an intention-to-treat basis. Adverse events were graded using the CTCAE Version 5.0. Ixazomib was given at a dose of 4mg orally weekly on days 1, 8 and 15 of a 28-day cycle. Lenalidomide was started at a standard dose of 15mg (Days 1-21) whilst dexamethasone was 40mg weekly.

Statistical analysis was performed using SPSS version 25. Approval for analysis and publication was obtained from the institutional review board at University College London whilst written consent was obtained from all patients in accordance with the Declaration of Helsinki.

Results

Forty two patients were identified (2 patients were excluded [1 declined follow up and 1 commenced treatment prior to review]) of which forty patients were evaluable for outcomes. Baseline characteristics are detailed in **Table 7.1**. Median time from diagnosis to commencement of IRd was 21 (range 5-132) months. All patients had received prior bortezomib. Patients received a median of 7 (range 1-36) cycles. Two patients only received one treatment cycle (1 death, 1 grade 3 maculopapular rash).

Haematological responses were assessed on an intention-to-treat basis; one patient was excluded from the response analysis due to missing data. Three month haematological responses were: CR – 8 (20.5%), VGPR – 8 (20.5%), PR – 7 (17.9%) and NR – 16 (41.0%). Six patients subsequently improved their response. Best responses were: CR - 10 (25.6%), VGPR - 8 (20.5%), PR - 7 (17.9%) and NR - 14 (35.9%) (**Figure 7.1**). Median time to response was 2 (range 1-9) months. In total, 94.1% of patients who achieved a deep haematological response (CR or VGPR) did so within 2 months. None of the twelve patients who had received prior lenalidomide achieved a CR at 3 months but 58.3% ultimately achieved a haematological response. Four patients had been refractory to prior lenalidomide and of these, 3 of 4 (75.0%) failed to respond to IRd. A further four patients were refractory to prior bortezomib of which two achieved a PR and two failed to respond to IRd. One patient was switched to melphalan-prednisolone after failing to respond to 2 cycles of IRd whilst the remaining three patients died within 8 months of commencing IRd.

Commented [CO(NWUHNT1]: define

Commented [CO(NWUHNT2R1]: Includes all patients irrespective of what happened to them

Commented [CO(NWUHNT3]: Around 65% response which is better than Len or Ixa alone

Commented [CO(NWUHNT4]: If no CR/VGPR within 2 months, unlikely to achieve it so consider early switch when need quick disease control - less likely in 3rd line setting

Commented [CO(NWUHNT5]: Still 58% response if prior Len (not Len refractory) but no CRs - Len refractroy 3/4 didn't respond

Table 7.1: Baseline characteristics of patients treated with ixazomib-lenalidomide-dexamethasone

	N(%)/Median(range)
Age, median (range)	66, 42-80 years
Male, N (%)	24 (60.0)
Disease Isotype	
IgG	18 (45.0)
Light Chain Only	15 (37.5)
IgA	5 (12.5)
IgM	2 (5.0)
Light chain isotype Lambda	31 (77.5)
dFLC (mg/L)	51.5 (0-100)
Mayo Stage at Presentation	
1	9 (22.5)
2	14 (35.0)
3A	14 (35.0)
3B	3 (7.5)
Organ Involvement	
Renal	28 (70.0)
Cardiac	26 (65.0)
Liver	11 (27.5)
Peripheral Nerve	1 (2.5)
Autonomic Nerve	6 (15.0)
Soft Tissue	12 (30.0)
Gastrointestinal	1 (2.5)
Baseline Organ Function	
Median eGFR ml/min per 1.73m^2	56 (>90-<15)
Proteinuria, g per 24h,	2.35 (0.1-16.4)
NT-proBNP, ng/L, median (range)	2445 (50-51661)
ALP, IU/L, median (range)	91.5 (13-1203)
Albumin, g/L, median (range)	35.5 (16.0-49.0)
Prior Lines of Therapy	
Median (range)	2 (1-4)
Bortezomib	40 (100.0)
Lenalidomide	12 (30.0)
ASCT	10 (25.0)
Lenalidomide refractory, N (%)	4 (10.0)

Figure 7.1: Haematological remission at 3 months and best response at any time after commencement of ixazomib-lenalidomide-dexamethasone. Demonstrates deepening of response in 6 patients.

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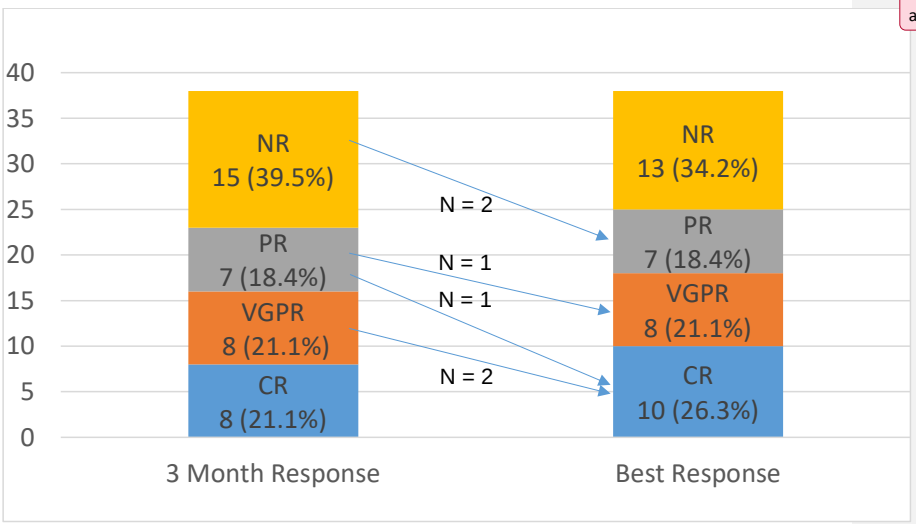
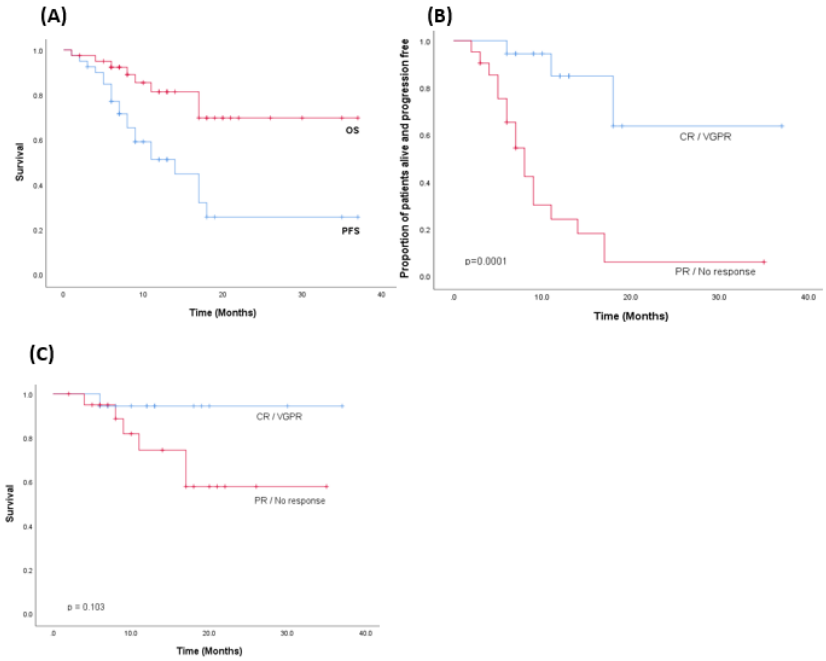


The median OS of the cohort was 29.1 months (95% CI: 24.4-33.8 months). Patients achieving a CR/VGPR (35.3 months [95% CI: 32.0-38.6 months]) did not survive significantly longer than those achieving a PR or non-responders (25.2 months [95% CI: 19.1-31.4 months]) (p=0.103). Median PFS was 17.0 months (95% CI 7.3-20.7 months). The median PFS for patients achieving CR/VGPR was 28.8 months (95% CI 20.6-37.0 months) and for ≤PR was 10.1 months (95% CI 6.0-13.6 months). (See **Figure 7.2 (A)-(C)**). In patients achieving any haematological response, OS was 32.5 months (95% CI 26.7-38.3 months). In contrast, OS was 16.2 months (95% CI 12.5-20.0 months) in non-responders (p=0.071). There was no significant difference in PFS (p=0.185) between patients who had received prior lenalidomide when compared with lenalidomide-naïve patients.

Commented [CO(NWUHNT7]: But longer PFS, maybe not long enough follow up or reflects fact that at the time those eligible may not have got CD38 Ab before 4th line

Figure 7.2 (A)-(C): Haematological response and survival in patients receiving ixazomib-lenalidomide-dexamethasone. (A) Overall survival of patients receiving ixazomib-lenalidomide-dexamethasone. (B) Estimated progression-free survival in relation to the haematological response. (C) Estimated overall survival in relation to the haematological response.



Organ responses were assessed at 6 months. The reliability of NT-proBNP to assess cardiac response whilst on treatment with lenalidomide remains unclear as immunomodulatory drugs have been associated with a rise in cardiac biomarkers (233). Consequently, the cardiac responses here need to be interpreted both with caution and with this caveat in mind as they may markedly under report cardiac organ response due to a paradoxical increase in NT-proBNP. Of twenty-six patients (65.0%) with cardiac involvement, 8 were not assessable for response (5 missing data and 3

Commented [CO(NWUHNT8]: Maybe should look at correlation with strain response in IMiD patients in future to see if impacts

baseline NT-proBNP <650ng/L). Of the remaining 18 patients, there was only one cardiac responder (5.6%). This patient achieved a CR within one month. There was cardiac progression in 8 (44.4%) cases – of these 4/8 (50.0%) were on IRd at time of response assessment. The remaining 10 (55.6%) did not respond. Ten patients (25.0%) had liver involvement based on alkaline phosphatase of which 7 (70%) were evaluable (2 not reached 6 months, 1 missing data); 4/7 (57.1%) progressed and 3/7 (42.9%) did not respond. Of the patients who demonstrated liver progression, 3 were non-responders and 1 achieved a PR.

Renal involvement was recorded in 28 (70.0%) patients. There was no significant difference in eGFR and creatinine levels between baseline and 6 months (p=0.328 and p=0.662 respectively). In patient with renal involvement, 3 month haematological responses were: CR – 8 (28.6%), VGPR – 6 (21.4%), PR – 2 (7.1%) and NR – 12 (42.9%). In this subgroup, the PFS and OS were 17.9 months (95% CI 11.8-24.0 months) and 31.6 months (95% CI 26.8-36.5 months) respectively. Thirteen patients (46.4%) were not evaluable for renal response: 3 not reached 6 months, 3 data missing, 4 on dialysis prior to IRd, 2 died and 1 baseline protein <0.5g/24h. Of 15 evaluable patients, 2/15 (13.3%) responded, 7/15 (46.7%) progressed and 6/15 (40.0%) were non-responders. Three patients progressed based on a creatinine increase and four patients based on worsening proteinuria.

Median follow up was 10.5 months (range 2-35 months). During the period of follow up, 8/40 (20.0%) patients died, 14/40 (35.0%) patients stopped treatment, 17/40 (42.5%) continue on IRd and 1/40 (2.5%) was lost to follow up. One patient stopped treatment after developing a grade 3 rash following the first dose of ixazomib. Of the remainder, 4/14 stopped due to grade 3/4 toxicity (2 infection, 1 renal, 1 bradyarrhythmia leading to cardiac arrest), 2/14 were palliated due to advanced

Commented [CO(NWUHNT9]: 1 cardiac responder and no liver responders. Haven't documented cardiac progression with haem response but typically <CR.

Commented [CO(NWUHNT10R9]: 2/15 renal responses

Commented [CO(NWUHNT11R9]: Part of this could be that it's 3rd line, less respond but also detected early so less scope for damage e.g. BNP rise that's reversible as often treat based on biochemical relapse

Commented [CO(NWUHNT12R9]: Plus median follow up 10.5 months

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disease with poor performance status and 7/14 had a suboptimal haematological response. Of these 7 patients, 4 commenced next line therapy (daratumumab x2, melphalan-prednisolone x1, pomalidomide-dexamethasone x1).

Adverse events are detailed in **Table 7.2**. Seven patients had a creatinine increase inclusive of 1 patient who required dialysis, which was classed as a serious adverse event. During treatment, serious AEs were seen in 35.0% of patients - there were 15 admissions in 12/40 (30.0%) patients: infection (6/15, 40.0%), fluid overload (5/15, 33.3%), cardiac arrhythmia (2/15, 13.3%), creatinine increase (1/15, 6.6%) and for a blood transfusion (1/15, 6.6%).

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Table 7.2: Adverse events in patients treated with ixazomib-lenalidomide-dexamethasone

Toxicity	Total adverse events n (%)	Grade 3-4 events n (%)
Thrombocytopenia	17 (42.5)	1 (2.5)
Fatigue	15 (37.5)	-
Constipation	10 (25.0)	-
Infection	9 (22.5)	6 (15.0)
Anaemia	9 (22.5)	2 (5.0)
Oedema	9 (22.5)	5 (12.5)
Neutropenia	9 (22.5)	-
Creatinine increase	7 (17.5)	1 (2.5)
Diarrhoea	6 (15.0)	-
Muscle / Bone Pain	6 (15.0)	-
Peripheral neuropathy	3 (7.5)	-
Nausea	3 (7.5)	-
Rash	2 (5.0)	1 (2.5)
Cardiac arrhythmia	2 (5.0)	2 (5.0)
Blurred vision	2 (5.0)	-
Insomnia	1 (2.5)	-

Discussion

This study demonstrates the efficacy of a novel triplet combination inclusive of an oral proteasome inhibitor, ixazomib, together with an immunomodulatory agent, lenalidomide, and dexamethasone in AL amyloidosis, which has not been previously reported. The data confirmed that this regimen has the potential to induce deep clonal responses with acceptable tolerability in the setting of relapsed disease.

Ixazomib, a next generation proteasome inhibitor, has shown promise in AL amyloidosis in a phase I study. However, a pivotal phase III study of ixazomib-dexamethasone vs. physician's choice was closed early after failing to reach its primary end-points (234), which raises questions regarding the efficacy of the doublet. Lenalidomide has been extensively used in AL amyloidosis in the relapse/refractory setting typically in combination dexamethasone but also with additional alkylators (109, 110, 235). Haematological response and survival data for other regimens including lenalidomide or ixazomib are documented in **Table 7.3**. Lenalidomide may be challenging to deliver in the context of renal dysfunction, especially in those patients with cardiac involvement, limiting the dose that can be delivered. In this cohort, the overall haematological rate (65.8%) compares favourably with prior studies of ixazomib monotherapy (52%)(119), ixazomib-cyclophosphamide-dexamethasone (57%) (230) and lenalidomide (51% (236) and 61% (229)). Furthermore, in this cohort almost half of patients achieved a deep haematological response (CR/VGPR) compared to less than a third with lenalidomide and 42% with ixazomib (120). Whilst the studies are not directly comparable, the data is encouraging and reinforces the view that more frequent deep responses are observed with the triplet combination as compared to the doublets.

Commented [CO(NWUHNT16]: Mechanism

Table 7.3: A comparison of previous studies of treatment combinations including lenalidomide and ixazomib.

Study	Chemotherapy	Patient No.	Haematological response (CR)	Median PFS	Median OS
Current study	Ixazomib-Lenalidomide-Dexamethasone	40	65.8 (26.3%)	17.0m	29.1m
Mahmood et al 2014 (122)	Lenalidomide-dexamethasone	84	61% (20%)	73% (2 yr)	84% (2 yr)
Kastritis et al 2018 (108)	Lenalidomide-dexamethasone	55	51% (5.5%)		25m
Kumar et al 2012 (235)	Cyclophosphamide-lenalidomide-dexamethasone	35	60% (11%)	28.3m	37.8m
Dinner et al (2013) (109)	Lenalidomide-melphalan-dexamethasone	25	58% (8%)	3.1m	58% (1 yr), Median NR
Hegenbart et al 2017 (110)	Lenalidomide-melphalan-dexamethasone	50	68% (18%)	25.1m	67.5m
Sanchorawala et al 2017 (119)	Ixazomib-dexamethasone	27	52% (9.5%)	14.8m	85% (1 yr)
Dispenzieri et al 2021 (120)	Ixazomib-dexamethasone	168	53% (26%)	-	-
Muchtar et al 2020 (230)	Ixazomib-cyclophosphamide-dexamethasone	35	57% (14%)	NR	NR

The clonal responses were rapid with median time to best response of 2 months. It appears that responses deepen with continuing therapy (similar to that documented with IRd in myeloma) (237) - 6 patients improved their response beyond 3 months including 2 patients who improved from PR to a CR and VGPR, respectively. Conversely, patients with a poor response at 3 months did not improve their responses significantly with continued therapy; non-response at 3 months should prompt consideration of switching to next line therapy. Encouragingly, patients who had prior exposure to lenalidomide (but not refractory) had good

responses whilst three out of four lenalidomide-refractory patients failed to respond. IRd appears to be a useful option of patients relapsing after prior lenalidomide treatment but may have a limited role in those who are lenalidomide refractory.

The progression-free survival for patients responding to IRd was excellent – at over 2 years. The PFS with ixazomib alone has previously been reported as 14.8 months in a small phase I study (119) but was not reached in the phase III study (120). The overall survival was also yet to be reached in a trial of ixazomib-cyclophosphamide-dexamethasone (although median follow up was just 4.4 months) (230). Lenalidomide combinations including cyclophosphamide and melphalan are reported to have a superior PFS of 25.1 (110) and 28.3 (235) months respectively; however, both trialled these therapies in new patients with limited exposure to other novel agent based therapies whereas, in this study, patients had a median of 2 prior lines of chemotherapy. A further study reporting on lenalidomide in combination with melphalan reported significantly worse outcomes (109) but did include 92% patients with cardiac involvement, a negative predictor of survival (22).

Commented [CO(NWUHNT17]: Interesting comparisons

The overall survival of our cohort was 29.1 months. This was not significantly different in those achieving a deep haematological response (CR/VGPR) when compared to those achieving ≤PR. The efficacy of 4th line daratumumab-based therapy together with the relatively short duration of follow up is thought to explain this. Further work is needed comparing both different ixazomib and lenalidomide-containing regimens in comparable patients to ascertain their relative efficacy.

The toxicity of this regime was manageable but not insignificant. Over 1/3 of patients experienced a serious adverse event, most commonly infection and fluid retention. However, these findings are not dissimilar from the grade 3/4 toxicity

reported with the individual drugs: 81% with ixazomib (119) and 27% with lenalidomide alone (229). Exact details of dose reductions and omissions from cycle-to-cycle are unavailable and remain a limitation of this retrospective study. Lenalidomide has been linked to renal dysfunction in AL amyloidosis (238) but there are no renal toxicities reported with ixazomib in AL amyloidosis (119). Kastritis and colleagues did report transient increases (to grade 1) in renal dysfunction and 5.5% developed acute renal failure requiring dialysis (236). In this study, 17.5% of patients developed acute kidney injury of which 1 patient required dialysis.

In summary, this data, reflecting the use of ixazomib-lenalidomide-dexamethasone in a real-world setting, gives a first-look at the efficacy and associated toxicities in patients with AL amyloidosis and prior bortezomib and lenalidomide exposure demonstrating encouraging deep haematological responses. This combination has the advantage of being administered orally on an outpatient basis. Patients achieving CR/VGPR have excellent PFS of 28 months. This study is limited by the small sample size and retrospective data collection. These results support further larger prospective studies to evaluate either IRd alone or in addition of a monoclonal antibody.

Chapter 8: The value of a rapid response to single agent daratumumab in improving progression-free survival in patients with relapsed/refractory systemic AL amyloidosis

This chapter is written in the context of my publication:

Rapid response to single agent daratumumab is associated with improved progression-free survival in relapsed/refractory AL amyloidosis. Cohen OC, Brodermann MH, Blakeney IJ, Mahmood S, Sachchithanantham S, Ravichandran S, Law S, Lachmann HJ, Whelan CJ, Popat R, Rabin N, Yong K, Kyriakou C, Shah R, Cheesman S, Worthington S, Hawkins P, Gillmore JD, Wechalekar AD. Amyloid. (2020), 27(3);200-205. Permission for use in my thesis obtained from the office of the publisher, Taylor and Francis.

Introduction

Systemic AL amyloidosis is characterised by the deposition of a monoclonal light-chain immunoglobulin in organs leading to, often critical, dysfunction (185). Whilst patient survival continues to improve, most patients relapse following initial therapy thus necessitating the use of further amyloid-directed therapy at relapse. Daratumumab is a monoclonal antibody (IgG1k), which targets CD38; an antigen expressed on malignant plasma cells (239). The use of daratumumab in amyloidosis has previously been examined in a number of case series documenting haematological response rates of 65-86% and rapid median times to response of 1-2.6 months (124, 125, 240-242). Furthermore the Boston Medical Center group have

reported a median time to first haematological response of just four weeks and a dFLC response rate of 90.5% after a single dose of daratumumab (242). Most recently, a multicentre study, by the European Myeloma Network, of daratumumab monotherapy in patients with stage IIIb amyloidosis demonstrated a 71% response rate with 18% achieving a complete haematological response but patient numbers were small (243). Here, the UK experience of single agent daratumumab for relapsed / refractory systemic AL amyloidosis is reported inclusive of an examination of the impact of the rapidity of the haematological response on survival outcomes.

Method

All patients treated with single agent daratumumab for relapsed/refractory systemic AL amyloidosis were identified from the databased at the UK NAC (2016-2019). The diagnosis of AL amyloidosis was confirmed by Congo red staining of tissue biopsy with confirmation of subtype by immunohistochemistry with specific antibodies or by mass spectrometry. Daratumumab was administered at standard doses of 16mg/kg weekly for 8 doses, fortnightly for 8 doses then monthly until disease progression.

Haematological and organ responses were defined as per consensus guidelines (167, 172). Given recent reports of the benefits of a deep reduction in dFLC on outcomes (104), we assessed absolute dFLC to identify patients who had achieved a dFLC <10mg/L (as previously published(104)). Adverse events were graded using the CTCAE Version 5.0. Overall survival was defined as the time from commencement of daratumumab to death from any cause whilst PFS was calculated from commencement of daratumumab to haematological progression, change in

treatment or death from any cause. All survival outcomes were calculated on an intention-to-treat basis.

Results

Fifty three patients, who received daratumumab monotherapy were identified. Median time from diagnosis to commencement of daratumumab was 32 (range 3-115) months. Full baseline characteristics are recorded in **Table 8.1**.

Table 8.1: Baseline Patient Characteristics at Time of Daratumumab Initiation

	N(%)/Median(range)
Age, median (range)	68 (42-85)
Male, N (%)	34 (64.2)
Disease Isotype	
IgG	31 (58.5)
Light Chain Only	14 (26.4)
IgA	5 (9.4)
IgM	2 (3.8)
IgD	1 (1.9)
Light chain isotype Lambda	36 (67.9)
dFLC, median (range) (mg/L)	78.9 (0.3-4897)
Bone marrow plasma cell (%)	16 (3-85)
Mayo Stage at Presentation	
1	11 (20.8)
2	19 (35.8)
3A	18 (34.0)
3B	5 (9.4)
Organ Involvement	
Cardiac	39 (73.6)
Renal	30 (56.6)
Liver	14 (26.4)
Soft Tissue	15 (28.3)
Peripheral Nerve	6 (11.3)
Autonomic Nerve	5 (9.4)
Gastrointestinal	4 (7.5)
Baseline Organ Function	
Median eGFR ml/min per 1.73m^2	51.5 (<15 – >90)
Proteinuria, g per 24h,	2.5 (0.1-16.8)
NT-proBNP, ng/L, median (range)	1962.5 (90-46412)
ALP, IU/L, median (range)	85 (17-516)
	39 (22-48)

Albumin, g/L, median (range)	
Prior Lines of Therapy Median (range) Bortezomib Lenalidomide ASCT	 3 (1-4) 49 (92.5) 44 (83.0) 13 (24.5)

Haematological responses were evaluable in 50 patients (2 low baseline dFLC <20mg/L and 1 death prior to response assessment). At 3 months, assessable responses were: CR – 19 (38%), VGPR – 14 (28%), PR – 9 (18%) and NR- 8 (16%) (**Figure 8.1**). Five patients with Mayo IIIb biomarkers, at baseline, were included in the study. In this subgroup, haematological responses were: CR – 2 (40%), VGPR – 2 (40%) and NR – 1 (20%). Over two-thirds of patients (36/53, 67.9%) received lenalidomide-based therapy immediately prior to commencing daratumumab. There was no significant difference between patients who received lenalidomide-based therapy immediately prior to daratumumab when compared to other agents (p=0.50). Haematological response by Mayo stage is documented in **Table 8.2**.

Figure 8.1: Haematological Response to single agent daratumumab by both international consensus criteria and dFLC response

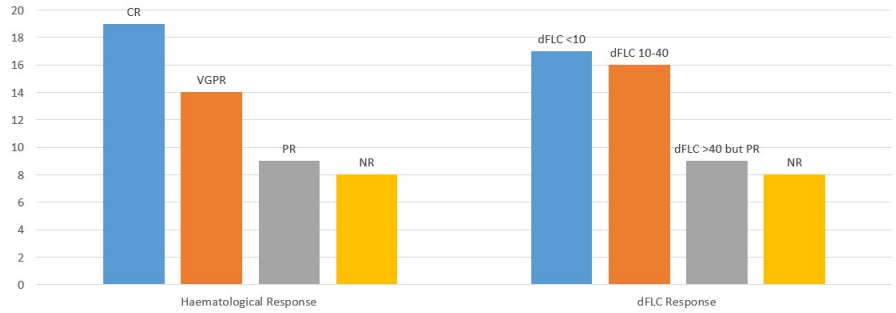
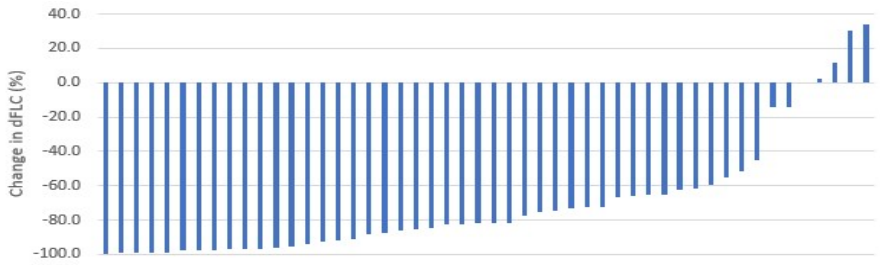


Table 8.2: Haematological Response to single agent daratumumab by Mayo Stage

	CR	VGPR	PR	NR	Total
Mayo I	3	1	3	3	10 (20%)
Mayo II	7	6	3	2	18 (36%)
Mayo IIIa	7	5	3	2	17 (34%)
Mayo IIIb	2	2	0	1	5 (10%)
Total	19 (38%)	14 (28%)	9 (18%)	8 (16%)	50 (100%)

Within the cohort, median time to response was 1 (range 1-6) month. Twenty-six patients (52.0%) responded within one month. Of these patients, 19/26 (73.1%) achieved a CR/VGPR compared to 12/15 (80%) who responded after 2 or 3 months. Only two patients achieved a haematological response beyond 3 months whilst two patients improved their response (1 VGPR to CR and 1 NR to PR). Seventeen (34%) achieved a dFLC <10mg/L (Figure 8.1). **Figure 8.2** displays the percentage reduction in dFLC showing some level of reduction in all but four patients. However, since initial haematological response assessment occurred at 1 month, we are unable to comment on the speed of response and its impact on outcome prior to the 1 month time point.

Figure 8.2: Percentage change in dFLC at 3 months post commencement of daratumumab monotherapy



Organ responses were evaluated 6 months post-initiation of daratumumab therapy. Of 39 patients with cardiac involvement, 16 were evaluable at 6 months (8 missing data, 6 not reached this timepoint, 5 baseline NT-proBNP <650ng/L and 4 NT-proBNP not assessable due to ESRF). Of these patients, 7/16 (43.8%) had a cardiac response, 4/16 (25.0%) progressed and 5/16 (31.3%) were non-responders. Of cardiac responders, 5/7 (71.4%) responded within 1 month and 6/7 (85.7%) achieved a CR. In patients with Mayo IIIb biomarkers, 2/5 (40%) lived beyond 6 months but neither was assessable for organ response (1 did not attend for re-assessment, 1 on dialysis). Whilst NT-proBNP was not evaluable for cardiac response in the patient on dialysis, there was evidence of an improvement in echocardiographic parameters with a change in longitudinal strain from -8.7% to -11.4%. This patient continues on daratumumab, and is maintained in a CR, eighteen months from commencement of therapy.

Commented [CO(NWUHNT18]: Better for dara than len, maybe Len increases BNP, ?need to assess by echo.

Thirty patients had renal involvement of which just 8 were assessable for organ response at 6 months (9 not reached this timepoint, 5 ESRF at baseline, 4 missing data and 4 baseline urinary protein <0.5g/24h). Two patients (25.0%) had a renal response, 1/8 (12.5%) progressed and 5/8 (62.5%) were non-responders. Finally, 14 patients had liver involvement inclusive of 8 evaluable for organ response at 6 months (1 not reached 6 months and 5 missing data). There were no patients who achieved a hepatic response. Of the remainder, 3/8 (37.5%) progressed and 5/8 (62.5%) were non-responders. The haematological responses in patients achieving any organ response were: CR – 7 (77.8%) and PR – 2 (22.2%). Of these patients, 7/9 (77.8%) achieved a haematological response within 1 month in comparison to 26/50 (52%) within the entire cohort. Within patients evaluable for a renal response, haematological response were evaluable in 5/6 (83.3%) non-responders (CR – 1

Commented [CO(NWUHNT19]: Small numbers

[20.0%], VGPR – 1 [20.0%], PR – 2 [50.0%] and NR – 1 [20.0%]). The high proportion of sub-CR responses within this small subgroup may explain the low renal response rate.

Patients were followed up for a median of 9 (range 2-35) months from the commencement of daratumumab therapy. Thirty-five (66.0%) patients continue on daratumumab, ten (18.9%) patients have died, four (7.5%) patients have stopped treatment and four (7.5%) patients have moved to next line therapy (addition of pomalidomide to daratumumab in 3 cases and addition of lenalidomide in the 4th case). Of the 10 patients who died, 5 died of progressive amyloidosis whilst 5 died whilst in a haematological response. Three of the four patients who had an immunomodulatory agent added improved their depth of haematological response. One patient stopped due to concerns regarding ongoing daratumumab maintenance in the setting of cardiac transplantation and the remainder due to inadequacy of haematological response as opposed to toxicity. Of the 10 patients who died, 1 patient was in CR but the remainder were in ≤PR (1 died prior to response assessment). Two patients stopped treatment with daratumumab following progression and were palliated prior to death.

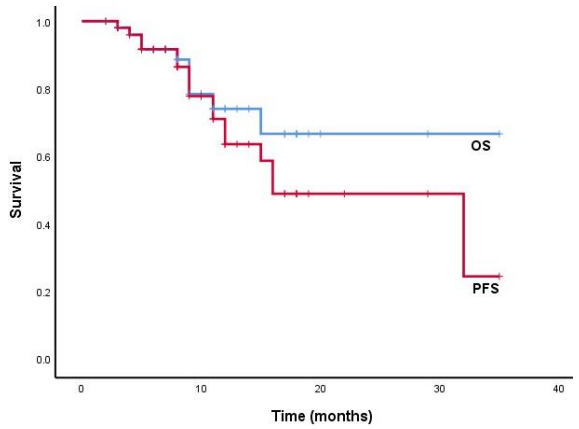
Median PFS was 19.9 months (95% CI 8.2-31.8 months) whilst median OS was not reached. Patients achieving a CR had a significantly longer median OS (not reached) compared to those in a lesser haematological response (median 22.7 months [95% CI 17.0-28.4 months]) (p=0.036) (Figure 8.2). Furthermore, patients achieving a rapid response (≤1 month) had a significantly longer median PFS (not reached) than those responding at a later time point (9 months [95% CI 5.8-12.2 months]) (p=0.013).

Figure 8.2: Survival in patients receiving daratumumab monotherapy

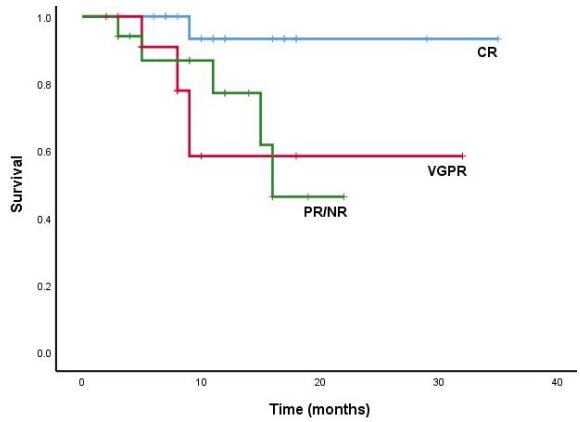
(A) Progression-free and overall survival from commencement of daratumumab monotherapy

(B) Overall survival of patients achieving a complete response vs. patients achieving a very good partial response vs. patients achieving a lesser response (partial response or worse).

(A)



(B)



Daratumumab was generally well tolerated. There were no therapy related deaths or grade 3-4 infusion reactions and no patient discontinued daratumumab due to toxicity. During the period of follow up, 6 (11.3%) patients were admitted to hospital (2 fluid overload, 2 falls [1 secondary to postural hypotension, 1 unexplained], 1 non-cardiac chest pain and 1 anaemia requiring blood transfusion in a patient with end-stage renal failure). Excluding the admissions listed, there was no grade III adverse events. The commonest grade I-II AEs are listed in **Table 8.3**. The nature of the infections listed were: tonsillitis, upper respiratory tract infection, lower respiratory tract infection (x2) and a urinary tract infection.

Table 8.3: Adverse events in patients on daratumumab monotherapy

Adverse event	Any grade, n (%)	Grade 3-4, n (%)
Infusion Reaction	7 (13.2)	0
Fatigue	6 (11.3)	0
Thrombocytopenia	6 (11.3)	0
Infection	5 (9.4)	0
Anaemia	5 (9.4)	1 (1.9)
Fluid Overload	4 (7.5)	2 (3.8)
Diarrhoea	3 (5.7)	0
Fall	2 (3.8)	2 (3.8)
Nausea	2 (3.8)	0
Insomnia	2 (3.8)	0
Hypertension	1 (1.9)	0
Blurred vision	1 (1.9)	0

Discussion

Daratumumab monotherapy is well tolerated and effective in relapsed/refractory systemic AL amyloidosis. The overall response rate was 84% in this cohort, which is consistent with previous literature (124, 125, 240-242). Crucially, a majority of patients treated in this study had received prior bortezomib (92.5%) and lenalidomide (83.0%) – the commonly used agents in the upfront setting. The response rates seen with daratumumab monotherapy appear to be superior to those achieved with other novel agents in the relapse setting such as ixazomib (53% (120, 127)), pomalidomide (46-61% (244)) and carfilzomib (63% (118)). Furthermore, the toxicity profile is manageable with grade III adverse events seen in just 11.3%, which compares favourably with alternative agents [ixazomib: 59% (127), carfilzomib: 71% (118)]. On pomalidomide therapy, discontinuation rates of 66-93% (244) are reported, whilst in this study, no patient discontinued due to documented toxicity.

Whilst rapid response to daratumumab monotherapy has been demonstrated (124, 125, 240-242), we show that time to reaching response is prognostic and confers a PFS advantage. In order to determine whether this translates to an overall survival advantage, a longer period of follow up is needed. Late responses are rare (only 2 patients beyond 3 months) indicating that a change in therapy should be considered early in non-responders.

Further work has examined the value of daratumumab as part of combination therapy. The Mayo group have published outcomes of 22 patients treated with daratumumab combination therapy (most bortezomib, pomalidomide or lenalidomide) demonstrating an 88% overall response rate (125). Subsequently, the ANDROMEDA study found that patients receiving daratumumab in combination with bortezomib-cyclophosphamide-dexamethasone had a significantly higher complete

haematological response rate (53.3% vs. 18.1%) and that more cardiac and renal responses were seen in this cohort (128). Another Phase 1 trial examining the use of daratumumab in combination with ixazomib and dexamethasone has shown, in 18 patients, a haematological response rate of 100% and CR/VGPR rates of 72% (245). Whilst these combination regimens certainly hold promise, the significantly greater toxicity of any chemotherapy regimen in patients with AL amyloidosis compared to patients with multiple myeloma makes daratumumab monotherapy an appealing option for some individuals in this cohort.

Rapid haematological responses are known to improve overall survival in patients with Mayo IIIb AL amyloidosis; defined by a CR or VGPR at day 30 (88). A high proportion of organ responders achieve a prior haematological response in patients treated with daratumumab (246). Furthermore, patients achieving early organ response (within one year of normalisation of the sFLCs) have superior overall survival (247). In this cohort, 78.8% of patients achieving an organ response had achieved a prior rapid haematological response (within 1 month) in comparison to 52% of the cohort overall. This suggests that a rapid haematological response may impact the subsequent organ response but further assessment using greater patient numbers is required for validation.

In summary, daratumumab monotherapy is a safe effective therapy in patients with multiply-relapsed systemic AL amyloidosis. Responses are rapid, seen in 84% of patients and long lasting, especially in patients who respond within one month. Furthermore, 43.8% of assessable patients with cardiac involvement demonstrated an organ response making daratumumab an attractive option in this subgroup. In the era of daratumumab combination therapies, there remains a role for daratumumab monotherapy in patients with relapsed systemic AL amyloidosis.

Results Section 3: Organ transplantation in amyloidosis

Chapter 9: The impact and importance of achieving a complete haematological response prior to renal transplantation in AL amyloidosis

This chapter is written in the context of my publication:

The impact and importance of achieving a complete haematological response prior to renal transplantation in AL amyloidosis. Cohen OC, Law S, Lachmann HJ, Sharpley F, Ravichandran S, Mahmood S, Sachchithanantham S, Whelan CJ, Martinez De Azcona Naharro A, Fontana M, Hawkins PN, Gillmore JD, Wechalekar AD. Blood cancer journal. 10(5): 60. Copyright permission obtained for use in this thesis.

Introduction

AL amyloidosis is characterised by the misfolding of monoclonal light chain immunoglobulins, which deposit within visceral organs leading to damage (185). The prevalence of AL amyloidosis has increased significantly over time from 15.5 cases per million in 2007 to 40.5 cases per million in 2015, which constitutes a 12% annual percentage change (4). Renal involvement is a frequent manifestation of disease and approximately one third of affected patients will develop end-stage renal failure (157). Patients with proteinuria ≥5g/day, estimated glomerular filtration rate <50ml/min/1.73m^2 and renal progression at 3 months have the highest rate of

progression to dialysis within one year (157, 248). Increasing prevalence and longer survival have contributed to a rise in the number of patients requiring either RRT or renal transplantation.

Achieving a deep haematological response to therapy in AL amyloidosis with renal involvement, at least a VGPR (157) or a 90% reduction in dFLC (249), improves renal outcomes and survival. In patients who progress to ESRF, renal transplantation is associated with lower mortality, less risk of cardiovascular events and improved quality of life compared to RRT (250). Furthermore, a historical Spanish study reported that patients with AL amyloidosis on dialysis had higher morbidity and mortality rates than matched controls with ESRF from other causes (251) making RRT even less attractive in this population. In 2010, the UK NAC reported a median OS of 6.5 years and graft survival of 5.8 years (151) in patients with AL amyloidosis who underwent renal transplantation, whilst a more recent report from the Boston Medical Center documented an impressive 10.5 year OS with an 8.3 year median graft survival (150) perhaps reflecting advances in patient selection and available therapies over the last decade.

Appropriate patient selection is imperative from the perspective of both fitness to undergo transplantation and depth of haematological response. Assessment of HR in patients with ESRF provides a challenge due to the polyclonal increase in serum free light chains that occurs in renal failure (252). We report the outcomes of patients who underwent renal transplantation for AL amyloidosis over the last 15 years at the UK National Amyloidosis Centre and examine the HR in these patients using both pre- and post-transplant light chains to assess their impact on response assessment and survival.

Methods

We identified all patients who had undergone renal transplantation for AL amyloidosis from the database of the UK NAC between 2005 and July 2019. Patients transplanted prior to 2005 were excluded given the marked changes in treatments and thus outcomes in earlier years. The diagnosis of AL amyloidosis was confirmed by central review of histological material inclusive of Congo red staining of the renal biopsy with demonstration of characteristic birefringence under cross-polarized light. The amyloid subtype was subsequently confirmed by immunohistochemistry with specific antibodies, or by mass spectrometry (161). Data collected for each patient consisted of a detailed baseline assessment inclusive of renal parameters, other organ involvements and sFLC measurements prior to and immediately post-renal transplantation.

Haematological responses prior to renal transplantation were defined as per published literature (54). In brief, complete response was defined by the absence of a monoclonal protein detectable in either serum or urine in addition to a normal sFLC ratio. In the absence of a CR, a partial response was defined as a dFLC concentration <50% of the pre-treatment value. A non-responder had a dFLC >50% of the pre-treatment value. Finally, a very good partial response was defined as an absolute dFLC <40mg/L, in the absence of a CR. Haematological response was also assessed using a wider sFLC normal range (0.37-3.1) (253) to define CR, to better account for the polyclonal sFLC increase in ESRF. In the post-transplant setting, haematological response was assessed as per international consensus criteria (167). Organ involvement was determined as per consensus guidelines (172). Given that LV mass increases and is prognostic in uraemic cardiomyopathy (254), various LV wall

thickness parameters were assessed. However, above 14mm the patient numbers were too small to generate meaningful results.

Progression free survival was defined as time from renal transplantation to haematological progression or death whilst OS was calculated from the date of renal transplantation to death from any cause. Time to ESRF was calculated from time of diagnosis to commencement of RRT or renal transplant. Renal graft survival was calculated as time from renal transplantation to recurrence of end-stage renal failure. Death without graft failure was censored. SPSS version 25 (IBM Corporation, Armonk, NY, USA) was used to perform the analysis. Survival data was analysed via the Kaplan-Meier method with two-sided p-values generated using the log rank test.

Results

Fifty patients with AL amyloidosis who underwent renal transplantation were identified. Five patients had renal transplants for reasons unrelated to amyloidosis (prior to their diagnosis), 4 patients were lost to follow up and 1 patient had a renal allograft for AL amyloidosis abroad prior to first presentation to the NAC. Forty patients were evaluable for outcomes. Baseline patient characteristics are summarised in **Table 9.1**. Fifteen patients (37.5%) had renal involvement only. The remaining patients had other multi-organ involvement including 13/40 (32.5%) with cardiac involvement. At presentation, 12 (30%) were in ESRF and a further 24 (60%) developed ESRF prior to renal transplantation whilst 4 (10%) were transplanted pre-emptively. The renal stage of patients not on dialysis at diagnosis were stage I – 2 (5%), stage II – 8 (20%), stage III – 8 (20%) and stage IV – 10 (25%). Median time from diagnosis to ESRF was 15 months (0-115 months) and from RRT to renal allograft was 28 months (3-83 months). Prior to transplantation, patients received a median of 2 prior lines of

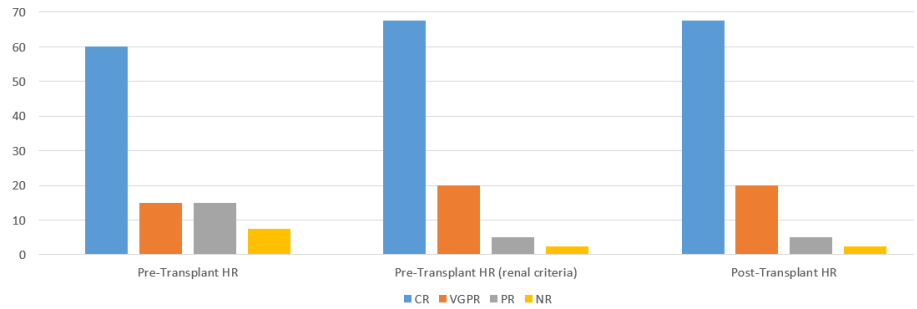
chemotherapy (range 1-4). Twenty patients (50%) received cadaveric transplants whilst the remainder had a live donor.

Table 9.1: Baseline characteristics of patients who underwent renal transplantation

Characteristic	N (%) / median (range)
Age	53.5 (38-69)
Male ECOG	24 (60) 1 (Range 0-3)
Disease Isotype IgG Light Chain Only IgA IgM	 20 (50) 16 (40) 2 (5) 2 (5)
Lambda restricted dFLC-R	28 (70) 112.7 (5.0-708.9)
Extra-renal Involvement Cardiac Liver Peripheral Nerve Autonomic Nerve Gastrointestinal Spleen Soft Tissue	 16 (40) 21 (52.5) 3 (7.5) 5 (12.5) 1 (2.5) 31 (77.5) 3 (7.5)
Baseline Organ Function Creatinine Median eGFR ml/min per 1.73m^2 Proteinuria, g per 24h NT-proBNP, ng/L ALP, IU/L Albumin, g/L	 199μmol/L (69-756 μmol/L) 27ml/min 8.95g/24h (0.4-19.7g/24h) 1915.5ng/L (76-69999ng/L) 78 IU/L (19-1384 IU/L) 28g/L (12-46g/L)
Prior Lines of Therapy ASCT	2 (1-4) 10 (25)

Haematological responses at renal transplantation were: CR – 24 (60.0%), VGPR – 6 (15.0%), PR – 6 (15.0%) and NR – 3 (7.5%). One patient was excluded as their light chains were not evaluable. Median time from haematological response to renal transplant was 29 months (range 0-93 months). No patient received chemotherapy between the pre- and post-renal transplant sFLC measurements. Based on post-renal transplant light chains, 7 patients (17.5%) had their haematological response re-classified, of which 6/7 (85.7%) were assigned an improved response. Haematological responses immediately post-transplantation were CR – 27 (67.5%), VGPR – 8 (20.0%), PR – 2 (5.0%) and NR – 1 (2.5%). One patient died before these readings were taken. When a wider sFLC normal range, used for patients with ESRF (253), was applied to pre-transplant response assessment, there was no change in the assigned depth of response following renal transplantation (see **Figure 9.1**).

Figure 9.1: Haematological response prior to and following renal transplantation. Pre-renal transplantation haematological responses were assigned using both the standard and renal-adapted haematological response criteria



Outcomes were studied using dFLC value (<10mg/L or <20mg/L), percentage reduction (>90%) and involved free light chain percentage reduction (>70%). There were no significant differences between responders and non-responders using pre-transplantation sFLC results. Using post-transplant clonal markers, OS was significantly better in patients with a deeper response assessed either as standard CR or dFLC <20 or <10 mg/L, iFLC reduction of >70% or dFLC reduction of >90% compared to ≤VGPR or a lesser depth of d/iFLC response, respectively (**Table 9.2**).

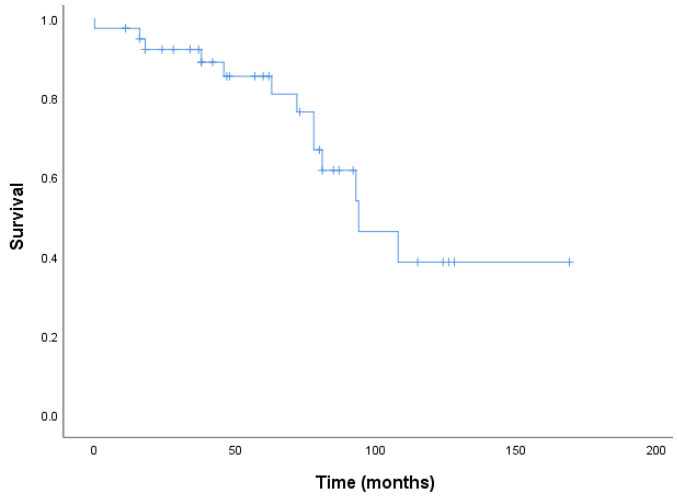
Table 9.2: Overall survival pre- and post-renal transplantation based upon traditional haematological criteria, absolute and percentage dFLC reduction and iFLC percentage reduction

	Overall survival based on pre-transplant haematological response assessment	P value	Overall survival based on post-transplant haematological response assessment	P value
CR	123.9 (72.8-143.2) months	0.015	137.8 (113.3-161.6) months	0.0001
VGPR or worse	70.4 (38.4-117.6) months		58.9 (34.4-91.6) months	
dFLC >90% reduction	114.4 (47.6-140.4) months	0.602	133.5 (109.3-158.4) months	0.0001
dFLC <90% reduction	92.9 (63.9-122.1) months		64.4 (41.9-114.1) months	
dFLC <10mg/L	Nil Patients		137.8 (112.4-163.2) months	0.0001
dFLC >10mg/L			68.5 (58.7-97.3) months	
dFLC <20mg/L	108.9 (70.4-117.6) months	0.561	122.2 (98.5-145.9) months	0.0001
dFLC >20mg/L	87.9 (71.8-114.2) months		58.6 (27.5-98.5) months	
iFLC >70% reduction	108.0 (61.7-124.3) months	0.953	118.9 (85.2-130.8) months	0.008
iFLC <70% reduction	86.0 (65.8-106.2)		64.7 (53.4-102.6 months)	

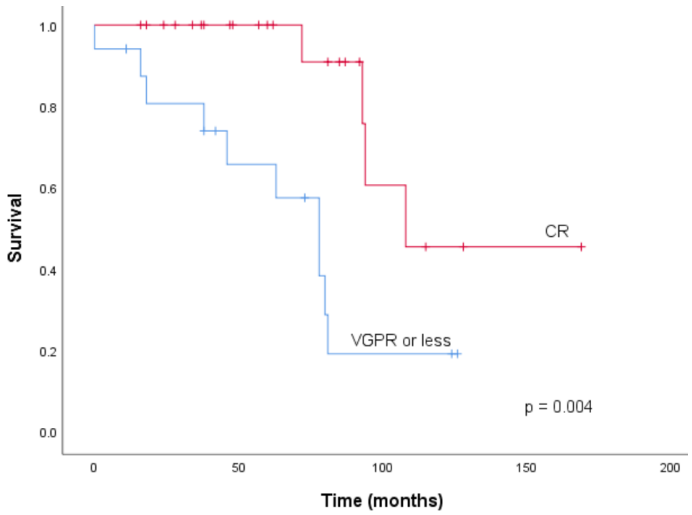
From renal transplantation, patients were followed up for a median of 8.9 years (2.2-19.3 years). During the period of follow up, 13 (32.5%) patients died. One patient died within a few days of renal transplantation following a hypotensive event and was known to have Mayo Stage III cardiac amyloidosis. Of the remaining deaths, 5 patients died in relapse, 5 died whilst in haematological remission and 2 are unknown. One patient developed a post-transplant lymphoproliferative disorder but there were no other secondary malignancies. From renal transplantation, haematological PFS was 6.9 years (95% CI 5.1-8.7 years) and median OS was 9.0 years (95% CI 5.5-10.1 years). Patients who achieved a CR based on pre-transplant sFLCs achieved a markedly higher PFS (8.5 years; 95% CI 5.7-11.4 years) (p=0.024) and OS (10.3 years; 95% CI 6.1-11.9 years) (p=0.015) (Figure 9.2). In contrast, patients achieving a pre-transplant CR or VGPR, did not have significantly different PFS (p=0.293) or OS (p=0.106) than those patients who were given a renal transplant in a lesser HR (PR/NR). Prior autologous stem cell transplantation did not impact survival. There was no difference in survival between those who died in relapse and those dying or other causes (p=0.360).

Figure 9.2 (A)-(B): Survival in patients who underwent renal transplantation (A) Overall survival in patients who underwent renal transplantation (B) Overall survival in patients who underwent renal transplantation by traditional haematological response

(A)



(B)



On univariate analysis (**Table 9.3**), source of renal transplant (live vs. cadaveric) and number of prior lines of chemotherapy had no significant impact on survival. Cardiac outcomes were assessed using the usual amyloid definition (LV wall >12mm) or a higher renal threshold (LV wall >13mm) given that increased LV wall thickness is both common and prognostic in end-stage renal failure (254). Above this level, patient numbers were too small for meaningful assessment. All patients with thicker LV wall (>12 or >13 mm) had a higher hazard ratio for poorer outcomes but it was significantly worse for those with LV wall >13 mm (median OS 9.7 years [7.8-11.7 years] vs. 3.6 years [0.7-6.6 years] for LV wall > or <13 mm respectively (p=0.01; HR 9.60 [2.08-44.23]). The NT-proBNP prior to renal transplantation had a worse hazard ratio for poorer outcomes but was not statistically significant.

Table 9.3: Factors impacting survival in patients who had undergone renal transplantation for systemic AL amyloidosis

Factor	HR (95% CI)	P value
Age >65 years	2.49 (0.82-7.54)	0.10
Baseline Cr >150	0.86 (0.30-2.50)	0.79
CKD Stage 5 at presentation	1.37 (0.46-4.12)	0.58
Performance Status	2.11 (0.89-4.98)	0.09
6 minute walk test	0.94 (0.84-1.06)	0.29
Organ involvement (3+)	0.96 (0.32-2.94)	0.96
Cardiac Involvement (>12mm)	2.22 (0.69-7.11)	0.17
Cardiac Involvement (>13mm)	9.60 (2.08-44.23)	0.01
Urine BJP at presentation	2.16 (0.63-7.45)	0.21
Baseline NT-proBNP >8500ng/L	4.67 (0.57-38.41)	0.09
NT-proBNP post-transplantation	1.83 (0.51-6.52)	0.36
Prior lines of chemotherapy	0.86 (0.50-1.5)	0.60

On an intention to treat basis, the median graft survival was 12.4 years (95% CI 10.7-14.2 years). There was 1 peri-operative death and 2 acute graft failures within 4 weeks of implantation (1 primary non-function with prolonged cold ischaemia time, 1 acute rejection). There was no significant difference between median creatinine post-transplant (128 µmol/L [44-382]) and at last follow up (113 µmol/L [54-775]) (p=0.42). On long term follow up, only one patient lost their graft and required initiation of RRT. Over the period of follow up, 9 (22.5%) patients required further chemotherapy of which one patient (11.1%) had evidence of increasing proteinuria. The remainder had evidence of haematological relapse only without organ progression. The treatments used at relapse were bortezomib-

cyclophosphamide-dexamethasone (6/9, 66.7%), ixazomib-lenalidomide-dexamethasone (2/9, 22.2%) and R-CHOP (rituximab-cyclophosphamide-doxorubicin-vincristine-prednisolone) followed by autologous stem cell transplantation for a diffuse large B cell lymphoma post-transplant lymphoproliferative disorder (1/9, 11.1%). None of these patients deteriorated in terms of chronic kidney disease stage or lost their renal graft during the period of follow up. There was no difference in graft survival (p=0.35) or OS (p=0.788) in patients who received chemotherapy following graft implantation.

Discussion

This study details the experience of renal transplantation for AL amyloidosis in the United Kingdom and demonstrates that the outcomes are encouraging in a carefully selected population. Outcomes are significantly better in patients with deep free light chain response and only 1 (2.5%) patient experienced graft failure secondary to recurrent amyloidosis. We highlight the challenge of accurately assessing haematological response in patients with ESRF, which results in a marked polyclonal increase in sFLCs (253); and the importance of post-renal transplantation sFLC measurements.

Patients selected for renal transplantation had an long median OS of 8.9 years following renal allograft, which is comparable to the 10.5 years reported by the Boston Amyloid group in their cohort (150) and improves upon data reported by our group previously (6.5 years (151)). The graft survival was excellent with either a live or cadaveric allograft and was not impacted by subsequent chemotherapy. Just 1 case of graft failure secondary to amyloidosis occurred over a median follow up of almost 9 years. Left ventricular hypertrophy is common and seen in nearly three quarters of

patients on dialysis (254). This was reflected in thicker LV walls of >13 mm predicting poorer survival. It is unclear whether this survival difference reflects a higher cardiac amyloid burden or more significant uraemic cardiomyopathy. This study highlights the importance of patient selection and pre-transplant cardiac status. The only peri-operative death occurred in a patient with Mayo stage 3 cardiac amyloidosis who presented with AL amyloidosis 4 years prior to renal transplantation and achieved a good haematological and cardiac response prior to renal transplant. Following this patient, our centre routinely undertakes functional stress cardiac testing (typically exercise stress echocardiography) in all patients with abnormal baseline echocardiograms considered for renal transplant assessment.

Within this patient cohort, only a CR pre-transplantation was found to have a survival advantage in contrast to previous work advocating either a CR or VGPR prior to transplantation. We found no difference in survival from renal transplantation in patients achieving VGPR or better when compared with lesser haematological responses in keeping with the Boston group. However, the Boston group did find a survival advantage in patients achieving VGPR when diagnosis, rather than renal transplant, was used at the starting point (150). This may reflect improved survival in patients with better responses to chemotherapy as opposed to the impact of HR at transplantation *per se*.

It is clear from the current and previous studies that a deep HR is of paramount importance and yet the current parameters used to assess haematological response in ESRF remain unreliable due to the polyclonal increase in sFLCs. The increase in polyclonal free light chains in CKD correlates with severity of renal failure and has led to a proposal to redefine the normal FLC ratio reference range in this patient population – from 0.26-1.65 to 0.37-3.1 (253). Implementing this reference range to define HR in

this cohort removes any re-classifications of HR post-transplant and confirms the problem of a large polyclonal free light chain component in the setting of ESRF. Mass spectrometry provides a novel method of identifying the monoclonal component of the sFLC but this is not yet widely available in clinical practice (80). Use of the renal FLC threshold for response assessment when patients are assessed for suitability for renal transplantation may be justifiable given the impact of haematological response on long term outcomes.

In the absence of improved criteria to assess HR prior to transplantation, we demonstrate the value of assessing HR post-transplant once the polyclonal element of the sFLCs are diminished to better predict patient outcome retrospectively. This resulted in 17.5% of patients having their depth of response re-classified (mostly VGPRs re-classified as CR's) after renal transplantation. Response classified post-renal transplant was found to be strongly predictive of survival based on various recognised methods of response assessment including traditional haematological response (CR, VGPR, PR, NR), absolute or percentage reduction in dFLC or percentage reduction in iFLC (255). In the pre-transplant setting, only categorisation into CR, VGPR, PR and NR was predictive of outcome albeit with less significance than post-transplant assessment by any method.

The literature on renal transplant outcomes in AL amyloidosis is relatively sparse and whilst this study is limited by its retrospective nature and relatively small patient numbers, we are able to demonstrate that graft failure secondary to AL amyloidosis is uncommon. We went on to contribute this data to a larger international study of renal transplant outcomes in AL amyloidosis, which included 237 patients from 5 countries. This study confirmed a similar median overall survival from renal

transplant of 8.6 years and found that median graft survival was longer in patients in a CR/VGPR (256).

Following renal transplantation in AL amyloidosis, patient survival is dictated by haematological response and LV wall thickness as opposed to graft failure. Both (HR and LV wall thickness) should be given careful consideration when determining whether a patient is suitable for renal transplantation. The polyclonal increase in sFLCs in ESRF pose a challenge in assessing depth of response prior to renal transplantation. Use of the renal FLC thresholds are more useful pre-transplantation. Use of the post-transplant light chains to re-classify response provides a better guide to patient outcome. Renal transplantation should be considered more often in patients with AL amyloidosis, predominant renal involvement and in end stage renal failure.

Chapter 10: The natural history and use of solid organ transplantation in patients with Apolipoprotein A-I amyloidosis

This chapter is written in the context of my publication:

The experience of hereditary apolipoprotein A-I amyloidosis at the UK National Amyloidosis Centre. OC Cohen, IJ Blakeney, S Law, S Ravichandran, J Gilbertson, D Rowczenio, S Mahmood, S Sachchithanantham, B Wisnioski, HJ Lachmann, CJ Whelan, A Martinez-Naharro, M Fontana, PN Hawkins, JD Gillmore and AD Wechalekar. Amyloid (2022) 29(4): 237-244. Permission for use in my thesis obtained from the office of the publisher, Taylor and Francis.

Introduction

Systemic amyloidosis refers to a group of heterogeneous disorders characterised by the misfolding of an abnormal fibrillar protein and its deposition within organs leading to dysfunction (198). AL amyloidosis remains the most common form, resulting from the production of light-chain immunoglobulin by a clonal cell population in the marrow, and accounts for approximately 55% of all cases (2). Hereditary forms of amyloidosis are usually autosomal dominant (albeit with variable penetrance) with specific clinical manifestations dependent on the mutated gene in question. Apolipoprotein A-I amyloidosis is the third most frequently occurring form of hereditary amyloidosis; hereditary transthyretin and fibrinogen amyloidosis being more common. Mutations in the genes encoding the APOAI protein can lead to deposition of amyloid fibrils within the heart, liver, kidneys, testis, nerves, larynx and skin with a mean age of onset of 58 years (257).

Apolipoprotein A-I is 28-Kda, non-glycosylated and the main apolipoprotein of HDL (38). Eighteen different amyloidogenic APOAI variants with resultant slowly progressive organ dysfunction are reported. A study of 253 carriers of the Leu75Pro variant found that 62% developed amyloidosis whilst 38% remained asymptomatic (257). Renal (39-46), cardiac (42, 47-51) and hepatic (39, 41, 42, 46, 52) involvement occurs in 11, 6 and 5 variants respectively. APOAI amyloidosis can present with amyloid deposition in unusual sites such as localised deposits in the aortic intima (with associated angina) (53) and palate (40) without co-existent amyloidotic organ dysfunction.

We present here the long term outcomes of a cohort of patients diagnosed with APOAI amyloidosis to document the natural history of the disease and the outcomes of patients who underwent single and combined solid organ transplantation.

Method

Patients

All patients with apolipoprotein A-I amyloidosis presenting between 1986 and 2017 who were reviewed at the UK National Amyloidosis Centre were included within the study. Diagnosis was based on histological proof of amyloid by Congo-Red staining and the finding of an underlying established or novel genetic mutation associated with APOAI amyloidosis. APOA1 amyloid fibril type was confirmed by immunohistochemistry with specific antibodies, or by mass spectrometry. Patients underwent a comprehensive assessment at the NAC on an annual basis inclusive of clinical assessment, blood monitoring of organ function, urine protein measurement,

echocardiography and ^{123}I-serum amyloid-P component scintigraphy as previously described (258). ^{123}I-SAP scintigraphy can visualise amyloid deposits in the liver, spleen, kidneys, adrenal glands and long bones but does not detect amyloid in other organs such as the heart or larynx.

Overall survival was calculated from date of presentation, as stated, to death from any cause whilst renal graft survival was calculated as time from transplantation to recurrence of end stage renal failure (deaths without graft failure were censored).

Statistical Analysis

Statistical analysis was performed using SPSS version 25. Approval for analysis and publication was obtained from the National Health Service institutional review board; written consent was obtained from all patients in accordance with the Declaration of Helsinki. Patients were censored at their last NAC visit. The Kaplan-Meier method was used to analyse survival outcomes using the log-rank test to compare differences between stratified Kaplan-Meier analyses.

Results

Fifty-seven patients with APOAI amyloidosis were included within the study. Thirty-three (57.9%) patients were male and 24 (42.1%) were female. Median age at presentation was 43 (17-75) years. There was no difference in age of presentation between males and females (p=0.30). Full baseline characteristics by APOAI mutation are detailed in **Table 10.1**. Overall, renal, hepatic and cardiac involvement was detected in 80.7%, 66.7% and 28.1% of patients respectively. Furthermore, 10.5% presented with neuropathy, 10.5% with laryngeal involvement and 3.5% with testicular involvement. At presentation, the median creatinine was 149µmol/L (range 51-718µmol/L) with an estimated glomerular filtration rate of

41.5ml/min (<15->90ml/min). Median urine protein was 0.25g/24h (0.1-12.8g/24h) and albumin 43g/L (21-51g/L) with just 7 patients presenting with nephrotic-range proteinuria (>3.0g/24h). The median alkaline phosphatase was 145.5 IU/L (44-554 IU/L). In terms of cardiac biomarkers, median NTproBNP was 355ng/L (21-14,478ng/L) and median Troponin T 10pg/ml (6-137pg/ml). The amyloid load, based on ^{123}I-SAP scintigraphy, was graded: small/equivocal: 12 (22.6%), moderate: 13 (24.5%), large: 16 (30.2%), and none: 12 (22.6%). ^{123}I-SAP scintigraphy was not performed in 4 patients. Median overall survival from presentation was 27.0 (21.3-32.7) years (**Figure 10.1**).

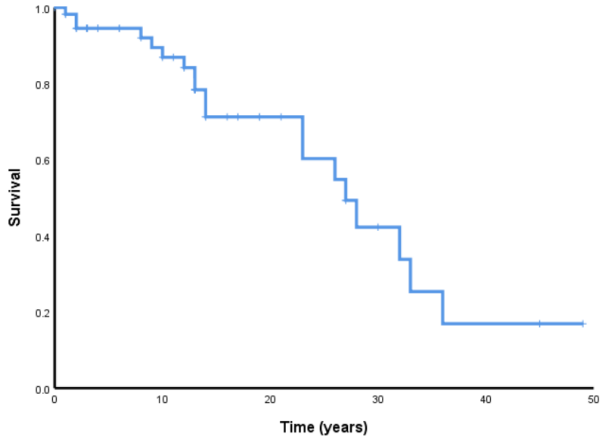
Patients with renal involvement by APOAI amyloidosis had significantly worse renal function at presentation (Creatinine: 159µmol/L vs. 103µmol/L, p=0.0004; eGFR: 37ml/min vs. 67.5ml/min, p=0.0001) but were not significantly more proteinuric (0.3g/24h vs. 0.15g/24h, p=0.06). Similarly, patients with hepatic involvement had a significantly higher ALP (244 IU/L vs. 77 IU/L, p<0.0001). Patients with cardiac involvement had greater NT-proBNP (1461ng/L vs. 271ng/L, p=0.006) values but baseline Troponin T was similar (14pg/ml vs. 11pg/ml, p=0.52).

Table 10.1: Baseline Characteristics of patients by apolipoprotein A-I variant

APOAI Variant	N (%)	Ethnicity	Age at presentation	Presenting Features	Organ Involvement	Amyloid Load
Gly26Arg	28 (49.1%)	Irish: 19 British: 9	42.5 (17-59)	CKD, Proteinuria, HTN (26), PN (1), Hepatic dysfunction (1)	Renal: 28 (100.0%%) Hepatic: 22 (78.6%) Cardiac: 5 (17.9%) Peripheral nerve: 5 (17.9%)	Large: 6 (21.4%) Moderate: 9 (32.1%) Small/Equivocal: 5 (17.9%) None: 5 (17.9%) Not done: 3 (10.7%)
Leu60Arg	7 (12.3%)	British	34 (23-67)	CKD, Proteinuria, HTN	Renal: 7 (100.0%) Hepatic: 7 (100.0%) Cardiac: 3 (42.9%) Gastric: 1 (14.3%)	Large: 4 (57.1%) Moderate: 2 (28.6%) Not done: 1 (14.3%)
Arg173Pro	6 (10.5%)	British	44.5 (32-66)	Hoarse voice (2), CHF (2), Asymptomatic screening (2)	Cardiac: 6 (100.0%) Laryngeal: 5 (83.3%) Renal: 1 (16.7%) Hepatic: 1 (16.7%) Choroidal: 1 (16.7%) Testicular: 1 (16.7%) Cutaneous: 1 (16.7%)	Small/Equivocal: 2 (33.3%) None: 4 (66.7%)
Trp50Arg	5 (8.8%)	British: 3 Polish Jewish: 2	42 (19-57)	CKD, Proteinuria, HTN	Renal: 5 (100.0%) Hepatic: 3 (60.0%)	Large: 2 (40.0%) Moderate: 1 (20.0%) Small: 2 (40.0%)
His155Metfs*46,delCc.535	2 (3.5%)	British	73 (71-75)	CKD, Proteinuria, HTN	Renal: 2 (100.0%) Peripheral nerve: 1 (50.0%)	Small: 2 (100.0%)
Leu64Pro	1 (1.8%)	Italian	56	Proteinuria, Oedema	Renal, Hepatic	Large
Leu90Pro	1 (1.8%)	British	75	CHF	Cardiac	None
Ala175Pro	1 (1.8%)	British	38	Dysphonia, Infertility	Larynx, Testis	None
Del 70-72	1 (1.8%)	British	21	Proteinuria, HTN	Renal, Retinal	Large
E70-W72	1 (1.8%)	British	33	Hepatic dysfunction	Renal, Hepatic	Large
F71Y	1 (1.8%)	Turkish	65	Proteinuria, HTN	Palate, Liver	Small
Gln172Pro	1 (1.8%)	Tanzanian	65	CHF	Cardiac	None
Glu34Lys	1 (1.8%)	Polish	27	Proteinuria, oedema	Renal, Hepatic	Large
Phe71Tyr	1 (1.8%)	British	47	Palatal lump	Palatal, Hepatic	Moderate

Figure 10.1 (A)-(B): Survival of patients diagnosed with hereditary apolipoprotein A-I amyloidosis. (A) Overall survival of all patients from presentation of apolipoprotein A-I amyloidosis. (B) Renal graft survival in patients who received a renal allograft with apolipoprotein A-I amyloidosis

(A)



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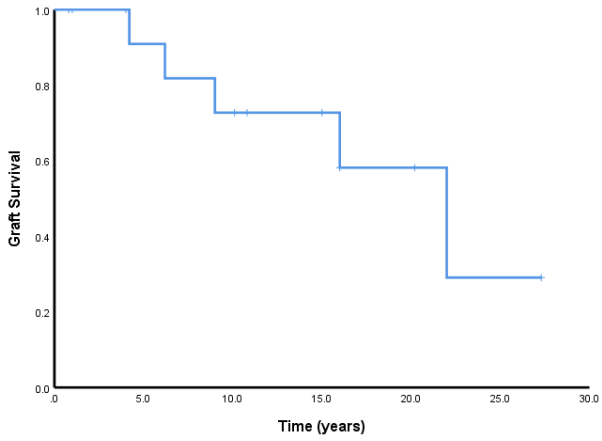


The most common variant detected was the APOA1 Gly26Arg mutation; 67.9% of these patients had Irish ancestry. At presentation, all patients had impaired renal excretory function, proteinuria and hypertension alone or in combination. At the time of referral to the NAC, the median eGFR amongst this group was 34ml/min (<15-75ml/min) with a median urinary protein of 0.2g/24h (0.1-4.0g). Ten patients were hypertensive at presentation. Patients carrying the Gly26Arg gene mutation with evidence of renal involvement had similar baseline creatinine (p=0.11) and eGFR (p=0.10) to patients with renal involvement and a different APOAI mutation but had significantly lower presenting urinary protein (0.15g/24h vs. 1.7g/24h, p=0.01) and higher albumin levels (44.5g/L vs. 40g/L, p=0.003). Whilst the only patient with Gly26Arg with nephrotic syndrome at presentation was already dialysis-dependent, none of the 6 other patients who were nephrotic at baseline had reached end-stage renal failure (Leo60Arg [2], Trp50Arg [2], His155Metfs*46, delCc.535 [2]). One patient with a Gly26Arg mutation presented with a debilitating peripheral neuropathy and subtle renal dysfunction. This patient had 4 affected brothers; one with predominant neuropathy and three with nephropathy. Similarly, all patients seen with Leu60Arg and Trp50Arg APOAI variants had evidence of nephropathy. All patients with an APOA1 Arg173Pro mutation had cardiac involvement. Only 2 out of 6 (33%) had symptomatic heart failure due to amyloidosis.

Forty-six patients presented with renal involvement. In this subgroup, median time to end-stage renal failure from presentation and date diagnosed was 19.0 (95% confidence interval [CI]: 7.5-30.6) years and 15.0 (95% CI: 10.0-20.0) years respectively. Twenty patients have reached ESRF and a further patient received a pre-emptive renal allograft with stage IV chronic kidney disease in combination with a liver transplant. At the time of writing, a further 10 patients underwent a kidney

transplant after commencing dialysis for ESRF, 2 died and 1 was listed for renal transplant but has subsequently been lost to follow up. Seven patients had a combined solid organ transplant (5 liver-kidney and 2 heart-kidney).

Of 11 patients who received an isolated renal transplant (3 live, 8 cadaveric), 5 patients have died (**Table 10.2**). The median survival from presentation was 32 (20.9-43.1) years and from renal transplantation was 19.7 (15.2-24.1) years. There was one early death due a cytomegalovirus infection within two months of transplant. Two patients died with documented amyloid recurrence within functioning grafts, 13 years after renal transplant. Two patients had amyloid recurrence in the renal allograft and proceed with a 2^{nd} renal allograft at 6 and 16 years post-transplant respectively – both had functioning grafts at the time of death. In the 6 living patients, 4 had functioning grafts (Patient / years' post-transplant: 16/4.0, 6/10.8, 12/15.0 and 1/27.3 years post-transplant) whilst 2 grafts had failed at 4.2 (Patient 14) and 9.0 (Patient 9) years respectively. None of the patients who underwent renal transplantation had significant cardiac amyloid. Two patients had evidence of possible early cardiac infiltration on imaging only. Within the cohort of patients who received a renal transplant and censoring deaths with a functioning graft, the graft survival of the cohort was median 16.0 (3.1-28.9) years.

Table 10.2: Characteristics of patients with hereditary apolipoprotein A-I amyloidosis undergoing solid organ transplantation

Patient No.	APOAI Variant	Organ(s) transplanted	Time to ESRF (years)	Amyloid load at Tx	Other organ involvement	Patient Status	Time from Tx to death/censor (years)	Cause of death	Graft with recurrent amyloid / time to recur	Amyloid load at death/censor
1	Del 70-72	Kidney	6.0	Large	Retinal	Alive	27.3	n/a	Yes	Unknown
2	Gly26Arg	Liver-Kidney	19.0	Large	Nil	Dead	18.0	Unknown	Yes	Small
3	Gly26Arg	Kidney	6.0	Moderate	Liver, PN	Dead	19.7	Unknown	Yes	Moderate
4	Gly26Arg	Kidney	8.0	Large	Liver	Dead	21.8	Unknown	Yes	Large
5	Leu60Arg	Kidney	13.0	Large	Liver	Dead	13.1	Liver failure	Yes	Unknown
6	Gly26Arg	Kidney	9.0	Large	Liver	Alive	10.8	n/a	No	Moderate
7	Leu60Arg	Heart-Kidney	1.0	Small	Liver	Dead	23.1	Unknown	Yes	Large
8	Arg173Pro	Heart-Kidney	10.0	None	Testis, Choroid	Dead	14.9	Progressive amyloidosis	Yes	None
9	Gly26Arg	Kidney	0	Moderate	Heart, Liver	Alive	9.0	n/a	No	Moderate
10	Gly26Arg	Liver-Kidney	9.2	Not done	Nil	Alive	10.1	n/a	No	Small
11	Trp50Arg	Liver-Kidney	8.0	Large	Nil	Alive	4.6	n/a	No	Not done
12	Gly26Arg	Kidney	4.1	Moderate	Nil	Alive	15.0	n/a	No	Small
13	Leu64Pro	Kidney	1.3	Large	Nil	Dead	13.2	Progressive amyloidosis	Yes	Not done
14	Gly26Arg	Kidney	1.1	Moderate	Cardiac	Alive	11.8	n/a	Yes	Large
15	Leu60Arg	Liver-Kidney	Pre-emptive	Large	Nil	Alive	1.0	n/a	No	Small
16	Trp50Arg	Kidney	1.0	Moderate	Liver	Alive	4.0	n/a	Yes	Large
17	Leu60Arg	Liver-Kidney	20.0	Large	Nil	Alive	17.6	n/a	No	Small
18	Trp50Arg	Kidney	10.0	Large	Liver	Dead	0.2	CMV	No	Unknown

Two patients received combined heart-kidney transplants. The time from transplant to death in patients 7 and 8 was 23.1 and 14.9 years respectively. Patient 7's renal graft failed after 22.1 years with multifactorial aetiology including recurrent amyloid. This patient received a live renal allograft thereafter but died suddenly (cause unknown) nine months later with a functioning graft. Patient 8 died with functioning grafts but had evidence of amyloid recurrence in both organs. One month prior to death, this patient had CKD stage 4 (Creatinine 240µmol/L and eGFR 24ml/min) and characteristic features of cardiac amyloidosis on echocardiogram with progressive worsening of left ventricular wall thickness (to 14mm) and 2-D longitudinal strain (to -10.7%) on annual imaging over a 5-year period prior to death.

Five patients received a liver-kidney transplant. One patient had a renal transplant performed 3 years after the orthotopic liver transplant whilst the other transplantations were simultaneous. There was one death in this group at 18 years post transplantation (cause of death unclear – both grafts were functioning well and death not felt to be due to amyloidosis). One patient required a second liver transplant due to hepatic allograft failure from strictures and recurrent infection. This patient's grafts are functioning well 3.9 years post-second liver transplantation. The remaining three patients are alive with functioning grafts and no evidence of recurrence at 10.1, 1.0 and 17.6 years post-transplantation respectively. Sequential ^{123}I-SAP scintigraphy performed in four out of the five patients with dual liver-kidney allografts, showed that there was evidence of marked amyloid regression following liver transplantation.

Discussion

This study reports the natural history and long-term outcomes of patients with hereditary apolipoprotein A-I amyloidosis inclusive of 14 different variants and represents the largest series of patients with the Gly27Arg variant reported to date. At the time of writing, this is the largest series reporting long term outcomes in transplanted patients with hereditary APOAI amyloidosis inclusive of 7 patients with combined solid organ transplants. We report an excellent median renal graft survival of 22 years from transplantation within this patient population.

The natural history of APOAI amyloidosis remains poorly documented due to a lack of large case series (**Table 10.3**). One series of 135 Italian patients exclusively examined the natural history of the Leu75Pro variant (257). This study documented an increasing penetrance with age finding that 98.7% of mutation carriers aged ≥80 years old had evidence of organ involvement. This study also found a mean age of onset of 58.1 years, consistent with other studies of the same variant (259, 260). In contrast, we found a younger age of onset across our patients of 43 years across multiple variants. An Irish study of 16 patients with APOAI amyloidosis also documented a younger age of onset of 48 years likely reflecting variation in age of symptom onset between genetic variants. Finally, the Italian study (257) found that age of onset was significantly lower in males than females (54.8 vs. 63.6 years) due to the earlier onset of testicular disease. A study of 10 patients with exclusive testicular disease confirmed this finding, documenting an average age of onset of just 35.7 years (261). Our study found no difference in age of presentation based on sex likely reflecting the small proportion of patients (3.5%) with documented testicular involvement.

Table 10.3: Published Apolipoprotein A-I Amyloidosis Series of ≥10 patients

Study	n	APOAI variants	Ethnicity	Median Age at Presentation	Organ Involvement by variant	Time from presentation to ESRF(years)	Organ transplantation
Gregorini *et al* 2015(257)	135	Leu75Pro	Italian	58.1	Renal (107/135) Hepatic (65/135) Testis (62/135)	Median age at ESRF 71.8 years	-
Pinney *et al* 2013(54)	16	Not documented	Not documented	-	Renal (16/16)	5.9	1 combined liver-kidney transplant and 13 kidney transplants
Traynor *et al* 2013(262)	16	Gly26Arg	Irish	48	Renal (15/16) PN (6/16)	9*	1 combined liver-kidney and 4 kidney transplants
Gillmore *et al* 2006(152)	10	Leu60Arg (3) Gly26Arg (2) Del70-72 (2) Trp50Arg (1) Leu64Pro (1) Arg173Pro (1)	English Irish Welsh Polish-Jewish Italian English	30.5	Renal (10/10) Cardiac (Leu60Arg, Arg173Pro) Hepatic (Leu64Pro, Trp50Arg, Gly26Arg)	8	2 combined liver-kidney, 2 combined heart-kidney and 6 kidney transplants
Scalvini et al 2007(261)	10	Leu75Pro	Not stated	35.7	Testis (10/10)	n/a	-
Gregorini *et al* 2005(260)	13	Leu75Pro	Italian	55	Renal (13/13) Hepatic (10/13) Cardiac (1/13)	-	-
Obici *et al* 2004(259)	13	Leu75Pro	Italian	56	Hepatic (12/13) Renal (9/13) Testis (2/13)	-	-

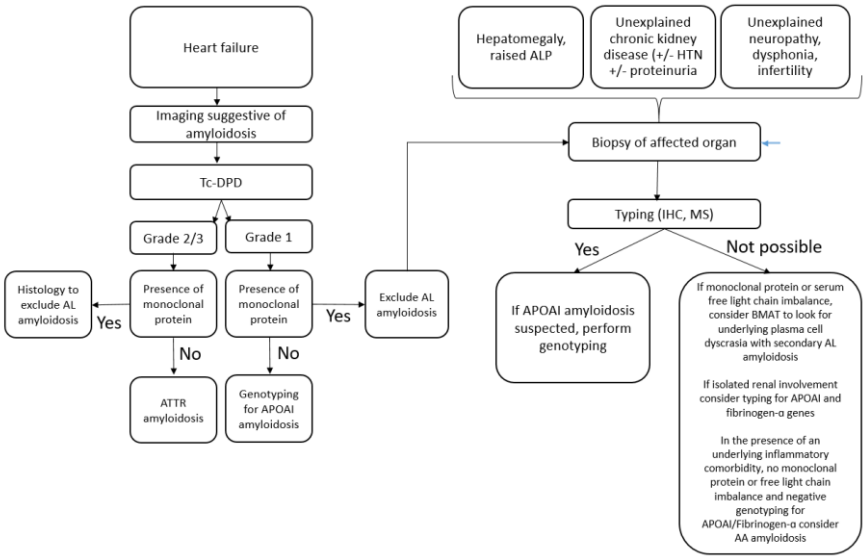
*Based on available data for 9 patients

In APOAI amyloidosis, organ involvement is dependent upon the disease variant. In other series, renal involvement has been documented in the majority of patients and, as such, is commonly viewed as the primary target organ of APOAI amyloidosis. In our series, renal involvement was universal in patients with the Gly27Arg APOAI variant. Patients slowly progress to ESRF (over median 19 years in this series) making early detection to optimise contributory factors such as hypertension of critical importance to prolong organ function. Hepatic involvement typically leads to hepatomegaly due to infiltration by amyloid but liver failure, usually by the 6th decade, is reported in a Spanish family with a Leu60Phe71 deletion / insertion Val60Thr61 variant within the APOAI gene. Within the heart, amyloid deposits lead to a progressive restrictive cardiomyopathy manifesting as congestive cardiac failure (48). Patients with the Arg173Pro, Leu90Pro and Gln172Pro variants universally presented with cardiac involvement. It is critical, not to discount APOAI amyloidosis as a potential diagnosis in cases with renal sparing. Other recognised manifestations, which were observed in this study and have previously been reported, include infertility (40, 49), hoarse voice/dysphonia (40, 48, 50, 51), polyneuropathy (39, 40, 45, 49) and cutaneous lesions (47, 48).

Silent organ dysfunction and resultant diagnostic delays pose a challenge in documenting the natural history of this rare disease particularly in patients without a clear family history. Furthermore, once patients present, symptoms are often non-specific leading to further diagnostic delays. In this series, patients waited a median of 3 years from presentation to referral for suspected amyloidosis. Apolipoprotein A-I amyloidosis should be considered in patients in whom amyloidosis is suspected on clinical or histological grounds especially in the context of either a suggestive family history or in the absence of a monoclonal protein in serum or urine with normal

serum free light chains. In such patients with isolated renal involvement, fibrinogen Aα-chain amyloidosis must also be considered. In AL, AA and fibrinogen-Aα amyloidosis with renal involvement, patients typically present with proteinuria, which is often in the nephrotic range (36, 157, 263). However, in APOAI amyloidosis, we found that patients with renal involvement generally had low-level proteinuria (median 0.3g/24h), which was not significantly higher than APOAI cases without renal involvement. In keeping with this, the Italian study of patients with Leu75Pro reported >0.5g/day of proteinuria in just 12% of affected patients (257). The presence of CKD secondary to suspected amyloidosis without significant proteinuria thus provides a clue to a diagnosis of APOAI amyloidosis. Furthermore, evidence of an underlying clonal dyscrasia should prompt both a bone marrow aspirate/trephine and target organ biopsy to exclude AL amyloidosis. A suggested diagnostic pathway for patients with suspected APOAI amyloidosis is shown in **Figure 10.2**.

Figure 10.2: Diagnosis of Apolipoprotein A-I amyloidosis in the absence of family history to guide diagnosis



Amyloid accumulation within an allograft together with continual progression in non-transplanted organs has made the use of organ transplants in this setting controversial historically. However, a recent study of transplant outcomes in patients with AL and AA amyloidosis demonstrated comparable death-censored graft survival in these patients compared to matched controls with renal allografts for diabetic nephropathy and adult polycystic kidney disease. Ten year death-censored graft survival in AL and AA amyloidosis was shown to be 93% and 78% respectively (264). Furthermore, graft survival in patients with APOAI amyloidosis has been shown to be significantly longer than graft survival in those with AL, AA or Fibrinogen Aα amyloidosis. In the largest study of renal transplant outcomes specifically in patients with APOAI amyloidosis, this work demonstrates a median graft survival of 22 years, which is superior to the 13.1 years previously reported (54). The slow rate of amyloid re-accumulation justifies renal transplantation in carefully selected patients with APOAI amyloidosis. Notably, no patient who received a renal allograft (with the exception of the 2 patients who received combined heart-kidney transplants) had cardiac involvement by amyloidosis at the time of transplantation. In AL amyloidosis, cardiac amyloid has been shown to increase the risk of death in patients undergoing renal transplantation (55) thus careful assessment of cardiac function prior to surgery is crucial. Both patients who received combined heart-kidney transplants benefitted from long-term functioning grafts again making combined transplant a viable option in suitable recipients.

In APOAI amyloidosis, the precursor protein is produced in the liver thus transplantation stops further production of the variant APOAI protein with historical studies demonstrating a fall in the concentration of the APOAI variant protein following liver transplantation (265). Similarly, we found evidence of amyloid

regression in all 4 patients who had pre- and post-transplantation ^{123}I-SAP scintigraphy performed occurring as early as 4.5 months post-transplantation. Our group had previously demonstrated marked regression in patient 17 (152) following liver transplantation (prior to the renal transplant whilst the patient remained on dialysis). At present, there are no drugs that can directly remove pre-deposited amyloid deposits from affected organs thus transplantation represents the only way to achieve this. Improvement in APOAI-associated peripheral neuropathy has also been reported following combined liver-kidney transplantation (152). However, given promising outcomes in patients with renal/cardiac grafts without liver transplantation, the routine use of liver transplantation in patients with APOAI amyloidosis cannot be justified in the absence of extensive hepatic amyloidosis with associated functional impairment.

In summary, we report here the outcomes of 57 patients with hereditary APOAI amyloidosis inclusive of 18 patients who underwent organ transplantation. APOAI amyloidosis is a slowly progressive disease with organ involvement dependent on the variant APOAI protein in question. Early recognition remains challenging. Outcomes of organ transplantation in selected patients are promising with excellent median graft survival of 22 years following renal transplantation seen in a mixed series of patients with single organ or combination transplants.

General Conclusions

The chapters presented in this thesis address key aspects of the diagnosis, management and prognostication of patients with amyloidosis. In particular, it explores the outcomes of a subgroup of patients with AL amyloidosis whose outcomes are especially poor and in whom treatment is particularly challenging, such as those with poor longitudinal strain on echocardiogram and poor function based on 6-minute walk testing.

Chapter 3 examines the value of screening biopsy in AL and transthyretin amyloidosis. In a group of 471 patients, the diagnostic sensitivity of abdominal fat aspirates, bone marrow trephines and gastrointestinal biopsies were evaluated. We show that a low-risk bedside abdominal fat aspirate has a 73.2% sensitivity for AL amyloidosis, which, when combined with a bone marrow trephine, which are performed routinely in patients with AL amyloidosis, yields a diagnosis in 82.9% patients. Furthermore, the diagnostic sensitivity of the abdominal fat aspirate in AL amyloidosis is even greater in patients with visceral organ involvement (cardiac [76.9%], hepatic [91.8%] and renal [78.0%]) who would traditionally be the patients where an invasive organ biopsy would be sought. Critically, these data highlight the value of abdominal fat aspiration in AL amyloidosis as a means to avoid a more invasive target organ biopsy. The full implications of this approach in terms of reducing cost, improving patient experience and reducing time to diagnosis require further evaluation. Conversely, the abdominal fat aspirate is just 27.3% sensitive in ATTR amyloidosis where a bone marrow trephine is invasive and not part of the diagnostic work up. Whilst many patients with ATTR amyloidosis will not require a biopsy since the validation of diagnostic criteria that are not reliant on histology (64), the study still identifies a value for abdominal fat aspirates in those cases when a biopsy is necessary. Whilst the diagnostic yield remains low, it is positive in over

one-quarter of patients who will be diagnosed in the absence of endomyocardial biopsy.

Chapter 4 focuses on the impact of longitudinal strain, measured on echocardiogram, upon haematological and traditional cardiac biomarker-based responses to therapy and overall survival. In a sample of 628 patients with cardiac AL amyloidosis, we demonstrate baseline strain worsened with increasing cardiac Mayo stage and identified a group of patients with a particularly poor prognosis based upon a longitudinal strain of ≥ -9.0%. Following chemotherapy, macrophage-led clearance of pre-deposited amyloid from affected organs may occur but the impact of this clearance on cardiac function and, specifically, the degree of strain improvement that would represent a meaningful change functionally had not previously been elucidated. In this chapter, we were able to demonstrate that any improvement in strain had a prognostic benefit but a change of -2.0% was deemed clinically important and accounted for a degree of inter- and intra-user variability that occurs when strain is measured. Importantly, an improvement in strain by -2.0% or more provides additional prognostic information over and above the traditional biomarker-based cardiac organ response criteria. We propose a new model to evaluate cardiac stage at baseline and organ response, following treatment, which incorporates longitudinal strain in addition to cardiac biomarkers.

Chapter 5 assesses the prognostic impact of the 6-minute walk test in AL amyloidosis. Whilst this test is validated in other diseases, particularly in the field of cardiology, data in AL amyloidosis is lacking. Furthermore, there is a clear unmet need for an objective determinant of functional capacity to assist in determining fitness for chemotherapy, the impact of treatment and subsequent functional improvement in patients achieving cardiac organ responses. Whilst other disease

groups have typically set a distance of 300m to define prognostic groups, we defined a new cut-point of 350 m, in 799 patients, that is specific to AL amyloidosis and independent of Mayo stage criteria. Patients who walked over 350m at baseline survived longer than those that did not. Again, we identified a new group, with a particularly poor prognosis (median 5 months), who were unable to attempt the test. This effect remained predictive of survival in patients with the most advanced disease – Mayo IIIb – who had an improved median survival of 59 months in those achieving ≥350m at baseline showing the heterogeneity of outcomes within these patients. This finding does require validation in larger studies as the sample size (n=31) in this sub-analysis was small. Based on serial changes in 6-minute walk distance, we showed that both the previously defined cut-point (33m) or amyloid-specific cut-point identified here (44m) predicted survival in patients with cardiac AL amyloidosis and were independent of haematological response. Whilst the optimal cut-point has yet to be determined, these findings suggest that walk testing represents a prognostic factor that could be considered as a trial inclusion/exclusion criterion and/or trial endpoint in studies of cardiac AL amyloidosis.

In chapter 6, we evaluate the role of serial health-related quality of life assessment in AL amyloidosis. In AL amyloid, the impact of organ dysfunction can be devastating whilst chemotherapy frequently leads to toxicities such as fatigue and neuropathy, which further impact quality of life. We use the SF36v2 questionnaire in 914 patients to show that patients report significant impairment in health-related quality of life even before treatment starts. Secondly, we find that there is a significant reduction in the physical component summary by 3 months, which improves, albeit not to back to baseline, by 12 months. Conversely, patients achieving a complete haematological response do see a mean improvement in

reported physical functioning, role physical and social functioning scores. Patients achieving a cardiac organ response saw some improvement in scores across all domains. Patients in lesser haematological responses did not see meaningful change in health-related quality of life domains and, moreover, those in a partial response or less saw a decline in reported quality of life across almost all domains. These findings, for the first time in AL amyloidosis, show that deep haematological and organ responses translate to improved quality of life. Measurements of health-related quality of life is a useful addendum to objective measures of a patient's physical fitness when evaluating treatment benefit and considering treatment options in AL amyloidosis.

Chapter 7 reports outcomes of a group of patients with systemic AL amyloidosis who were treated with ixazomib-lenalidomide-dexamethasone. As patients with AL amyloidosis live longer, the need for novel agents is increasing and whilst this combination is established in multiple myeloma, its use in the AL amyloid setting had not been previously reported. In the 3rd line setting, the overall response rate was 59% inclusive of a 20.5% complete haematological response rate. Responses were generally quick with a median time to best response of 2 months. Furthermore, the median progression-free survival was 17 months although this increased to 28.8 months in patients meeting criteria for at least a very good partial response. Whilst only limited organ responses were seen, this may relate to the fairly short period of follow up (median 10 months). The triplet combination was reasonably well tolerated. One patient did require dialysis due to decline renal function on treatment. Options for multiply relapsed patients with AL amyloidosis are limited and most therapies are licensed only in the multiple myeloma setting. These results show that this combination warrants further study in this setting.

Chapter 8 reports an analysis of a group of patients with AL amyloidosis treated with single agent daratumumab after 3 prior lines of therapy. Given the impact of amyloidosis on organ function and quality of life together with the toxicities of multiple lines of prior chemotherapy, the use of a tolerable but effective option for patients with AL amyloidosis in this situation is critically needed. There was an 84% overall response rate (38% complete response) in a cohort of 50 such patients. Again, responses were rapid with a median time to response of 1 month. There were cardiac organ responses seen in 43.8% and renal responses in 25.0% although numbers were small. Responses were durable in that 66.0% of patients continued on daratumumab therapy at a median follow up of 9 months with a progression-free survival of 19.9 months, which was not reached in those achieving a rapid response (within 1 month). Daratumumab was shown to be safe, effective and well tolerated with durable responses in a group of multiply treated patients and remains a viable option for some patients even in the era of daratumumab combination therapy.

Chapter 9 examines the outcomes of renal transplantation in AL amyloidosis. Prevalence and survival in AL amyloidosis is increasing with historical studies showing one-third of patients with renal involvement, a frequent manifestation in AL amyloidosis, develop end-stage renal failure. This study showed that the median graft survival was 12.4 years. On long term follow up (8.9 years), only one patient lose their graft due to amyloid recurrence. Patients in a complete haematological response at the time of transplantation had a significantly better progression-free and overall survival. We demonstrated the impact of the polyclonal increase in free light chains on haematological evaluation pre-transplant and suggest using a wider renal threshold for the serum free light chain normal range in these patients. Finally, we found that patients with a left ventricular wall thickness of >13mm had poorer

outcomes reinforcing that careful consideration should be given to, not only haematological response, but also degree of cardiac impairment prior to the decision to proceed with renal transplantation. With these caveats in mind, renal transplantation should be considered more frequently in suitable patients with systemic AL amyloidosis in end-stage renal failure.

Chapter 10 explores the use of solid organ transplantation in a cohort of patients with heritable Apolipoprotein A-I amyloidosis. This group of patients is heterogenous and different mutations lead to a different constellation of symptoms and organ involvement. Patients present with symptoms secondary to amyloid deposition within the heart, liver, kidneys, nerves, larynx, testis and skin. We report the natural history of the disease in 57 patients with a median age of presentation of 43 years. Renal, hepatic and cardiac involvement was documented in 80.7%, 66.7% and 28.1% of patients respectively. In the commonest variant seen within our cohort (Gly26Arg), seen in 67.9% of patients, all patients had renal dysfunction but level of proteinuria was low (median 0.15g/24h). This is noteworthy given that patients with other forms of renal amyloidosis typically present with nephrotic syndrome. Eighteen patients underwent renal transplantation, which in 7 cases was combined with a cardiac or liver transplant. Patients who underwent an isolated renal transplant had a median graft survival of 22 years whilst encouraging outcomes of combined organ transplants were also reported in cases of associates cardiac or liver failure. Given that APOAI is produced in the liver, the orthotopic liver transplantation introduced a non-amyloid producing liver, which over time resulted in regression of amyloid deposits in other organs on ^{123}I-SAP scintigraphy in all cases imaged (4/5 patients received a liver transplant and had serial imaging available). In an area where large case series are lacking, the natural history of APOAI amyloidosis is poorly

documented and this study contributes to the literature in this way whilst also representing the largest reported cohort of patients with APOAI amyloidosis undergoing solid organ transplantation.

Future studies

The combination of an abdominal fat aspirate and bone marrow trephine negated the need for invasive target organ biopsies in over 80% of patients. Further studies could include prospective evaluation of the volume of tissue obtained on abdominal fat aspiration to determine if this further increases the diagnostic sensitivity to avoid target-organ biopsies in even more patients. This principle can also be applied to ATTR amyloidosis although the diagnostic yield will be lower based on our findings.

We propose new staging criteria for use in patients with cardiac AL amyloidosis at baseline and following treatment to evaluate organ response. In the first instance, this requires validation. Our study looked at longitudinal strain only but similar studies could compare this to other echocardiographic parameters to determine whether the use of strain is indeed the optimal measure. Finally, further work should look at whether the criteria posed here could be modified to consider depth of cardiac organ response as opposed to the binary option of responders vs. non responders, which may be more informative. Similarly, we pose prognostic values for baseline 6-minute walk testing and change in walk testing at 12 months. Validation is particularly key to further elucidate the optimal cut-point for the level of improvement in walk testing at 12 months. We explored 44m as a value specific to this population whereas 33m had been posited previously in patients with pulmonary hypertension. The sensitivity and specificity of these values was similar in our study although 44m was marginally more sensitive and specific. Validation of the cut-points chosen in both the longitudinal strain and 6-minute walk testing studies is required in order to consider incorporation of these measures as inclusion criteria, exclusion

criteria and clinical end-points in trials of AL amyloidosis. Further work is planned to combine the cut-points posited in both the longitudinal strain and walk testing studies to evaluate the practicality and usefulness of combining the two factors into a single prognostic score also incorporating cardiac biomarkers. Given that not all centres have the resource to conduct the 6-minute walk test, consideration could be given to evaluating serial patient estimates of function to ascertain whether this could represent an alternative to walk testing in cases where conducting it is not practical.

Similarly, our health-related quality of life study identified changes in SF36v2 scoring post-chemotherapy, which were only improved in patients achieving a deep haematological response. The value used to define a clinically meaningful improvement in quality of life was not specifically designed with AL amyloidosis in mind and thus amyloid-specific cut-points could be investigated in future studies. We show that a deeper haematological response leads to improved quality of life scores but next plan to investigate the impact of change in these scores on survival to determine the independent prognostic impact of changes in health-related quality of life scores. Further work should also focus on ongoing collection of serial measurements of health-related quality of life to investigate the impact of relapse and subsequent lines of chemotherapy. This work could employ wearable technology, which would allow real time HRQoL measurements throughout the patient journey.

We examined the outcomes of ixazomib-lenalidomide-dexamethasone and daratumumab monotherapy respectively in patients with systemic AL amyloidosis. In both studies, the sample sizes were fairly small and larger studies would be of benefit to further investigate outcomes. This is particularly true for the combination of ixazomib-lenalidomide-dexamethasone where no other published studies of this

triplet combination's use in amyloidosis are available. In the case of daratumumab monotherapy, the impact of prior treatment with a monoclonal antibody therapy will be key as the combination of daratumumab-bortezomib-cyclophosphamide-dexamethasone may be approved for use in AL amyloidosis in the future given the encouraging results of the ANDROMEDA study. Examination of new treatment combinations, including novel therapies (e.g. venetoclax in patients with t(11;14) translocation) would be of great value particularly given that the use of quadruple therapy upfront will inevitably create of need for the introduction of therapies with different mechanisms of action to use in the relapse setting.

Renal transplantation in AL amyloidosis shows good outcomes and we have submitted our data for inclusion as part of a large international collaboration of renal transplant outcomes, which will serve to provide further information on outcomes and aid suitable patient selection. In the case of APOAI amyloidosis, the evidence base remains suboptimal and, particularly in the case of dual organ transplantation, data is incredibly sparse. Further publication of outcomes, case series and long term follow up of these patients will be critical to inform this field further. Ultimately, there is a need for anti-fibril therapies across all amyloid subtypes, which may negate the need for organ transplantation in many of these patients in the future.

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