Novel approaches in the prognostication, monitoring and management of light chain amyloidosis

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I, Faye Amelia Sharpley, confirm that the work presented in this thesis is my own. Where information has been derived from other sources. I confirm that this has been indicated in the thesis.

Abstract

Background

Systemic light chain (AL) amyloidosis is a rare disorder characterised by the production of abnormal clonal light chains, which mis-fold and deposit as amyloid fibrils within the tissues, with progressive organ dysfunction. Significant progress has been made in the field of AL amyloidosis, but improvements can still be made in the stratification of patients, the monitoring of patients who achieve a clonal remission and in the treatment of patients, both with autologous stem cell transplantation (ASCT) and at relapse.

Aims

- To explore the merits of mass spectrometry as a novel diagnostic technique.
- To analyse the features of Mayo stage 1 patients to help identify variables predictive for survival.
- To assess the outcome of patients treated with an autologous stem cell transplant and to compare outcomes with patients treated with standard first line chemotherapy.
- To analyse the outcomes of relapsed patients treated with the immunomodulatory drug pomalidomide.
- To explore the complications of treatment including reactivation of cytomegalovirus infection.
- To assess if amyloidosis can complicate solid organ transplantation.

Results and Conclusions

- Mass spectrometry can accurately identify and quantify a monoclonal light chain component, even in patients in a complete light chain response by current serological methods.
- The cardiac biomarker N- terminal B-natriuretic peptide still has a prognostic value in Mayo stage 1 patients.
- ASCT remains a safe and effective treatment with outcomes comparable to the large American transplant centres, but with no survival benefit over standard first line treatment.
- Pomalidomide is a therapeutic option for multiply relapsed AL amyloidosis patients but responses are not as sustained nor as deep in the real-world setting.
- There is a substantial risk of cytomegalovirus reactivation in patients treated with bortezomib
- Solid organ transplantation appears to be a genuine risk factor for the development of both AL and AA amyloidosis.

Impact Statement

The discovery and insights presented in this thesis offer benefits inside and outside the academic field of AL amyloidosis.

- The importance of cardiac biomarkers even in patients with Mayo stage I AL amyloidosis in chapter four has the potential to shake the AL amyloidosis community, prompting a re-evaluation of international guidelines defining cardiac involvement and the imaging methods used.
- The novel mass spectrometry technique presented in chapter three holds the promise for future scholarship/ research. The Binding Site Group are currently analysing samples from a larger cohort of UK NAC ALchemy patients and I hope others will continue this project.
- The development of amyloidosis post solid organ transplantation, described in chapter nine, has already sparked interest from myeloma academics, given that this is a previously unrecognised, yet important, phenomenon which requires further evaluation and assessment. The impact of this chapter also has wider reaching implications for those within the transplant research field.

This thesis will also influence practice outside academia, in the commercial field and also in current clinical practice:

- The novel mass spectrometry technique outlined in chapter three has potential clinical and commercial benefit. The ability to detect and monitor a pathogenic monoclonal light chain component in patients who are seemingly in a complete clonal remission will completely change clinical practice and offers an alternative from current methods of monitoring minimal residual disease, which rely upon invasive bone marrow sampling.
- Chapter five highlights how autologous stem cell transplantation has become an increasingly safe treatment over time, but chapter six compares outcomes of stem cell transplantation to conventional chemotherapy and warns that there may be no survival benefit. This may evoke a shift away from stem cell transplantation and may prevent unnecessary patient deaths and improve the quality of life for patients with this rare and difficult to manage condition.

I have taken steps to ensure that the impact of this thesis is realised, by disseminating my work through publication. I have submitted abstracts of the data presented in this thesis to regional, national and international meetings including the International Amyloid meeting in Japan and the American Society of Haematology meeting in San Diego. My abstracts have been accepted as poster presentations, in all cases, and three of the seven posters have qualified for abstract awards. I have made oral presentations of the work outlined in chapter four, on re-defining Mayo stage 1 disease, at the UK- National Amyloidosis Forum meeting and on chapter seven, on the use of pomalidomide, at the UCL research retreat. In writing this thesis

I have collaborated with academics in Italy and with the commercial Binding Site Group, in Birmingham, and I have held two amyloidosis patient support group sessions in both Belfast and Ascot, raising my own profile and that of the National Amyloidosis centre.

In conclusion this research thesis potentially has profound and far reaching impact, in the academic and non-academic communities, with the potential for immediate and long-lasting change to our understanding and clinical treatment of AL amyloidosis.

Ethical Approval

All data included in the clinical research studies described in this thesis was with informed consent in accordance with the Declaration of Helsinki. Written consent was given by patients on attendance at the National Amyloidosis Centre and the consent form approved by the Hospital Ethics Committee (REC Ref 06/Q0501/42).

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Publications arising from this thesis

Appropriate permissions from all journals has been obtained for the use of the article or figures in this thesis.

A novel mass spectrometry method to identify the serum monoclonal light chain component in systemic light chain amyloidosis. Sharpley FA, Manwani R, Mahmood S, Sachchithanantham S, Lachmann HJ, Gillmore JD, Whelan CJ, Fontana M, Hawkins PN, Wechalekar AD, *Blood Cancer Journal* **9**, 16 (2019). https://doi.org/10.1038/s41408-019-0180-1

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A twenty-four year experience of autologous stem cell transplantation for light chain amyloidosis patients in the United Kingdom

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Oral and Poster Presentations

Real World outcomes of pomalidomide for treatment of relapsed light chain amyloidosis. International Society Amyloidosis conferences in Japan. Bursary winner. Poster Presentation 2018.

Real World outcomes of pomalidomide for treatment of relapsed light chain amyloidosis. UCL Research Retreat. Oral presentation 2018.

Variables predictive of poor prognosis in patients with systemic

light chain amyloidosis. EHA Stockholm. Poster Presentation. 2018.

A novel mass spectrometry method to identify the serum monoclonal light chain component in systemic light chain amyloidosis. ASH San Diego. Poster Presentation. 2018.

Light chain amyloidosis as a rare complication of solid organ transplantation. BSH Glasgow. Bursary winner. Poster Presentation. 2019.

Light chain amyloidosis as a rare complication of solid organ transplantation. UCL Research Retreat. Poster Presentation. 2019.

A twenty-four year experience of autologous stem cell transplantation for light chain amyloidosis patients in the United Kingdom. EHA Amsterdam. Bursary winner. Poster presentation. 2019.

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Abbreviations

AA	Systemic amyloid A amyloidosis
AApoAI	Hereditary apolipoprotein AI amyloidosis
AApoAII	Hereditary apolipoprotein AII amyloidosis
$A\beta_2m$	Dialysis associated amyloidosis
ACys	Cystatin C amyloidosis
AFib	Hereditary fibrinogen A α-chain amyloidosis
AGel	Gelsolin amyloidosis
AH	Immunoglobulin heavy chain amyloidosis
AL	Light chain amyloidosis
ALect2	ALECT2 amyloidosis
ALys	Hereditary lysozyme amyloidosis
ALP	Alkaline phosphatase
ANS	Autonomic nervous system
ASCT	Autologous stem cell transplantation
ATTR	Transthyretin cardiac amyloidosis

BEAM	Carmustine, etoposide, cytarabine, melphalan
BMDex	Bortezomib, melphalan, dexamethasone
BMT	Bone marrow trephine
BP	Blood pressure
CAPS	cryopyrin associated periodic syndromes
CI	Confidence interval
CMR	Cardiac magnetic resonance imaging
CMV	Cytomegalovirus
CPHPC	(R-1-[6-[R-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid)
CR	Complete clonal response
CRP	C-reactive protein
CTS	Carpal tunnel syndrome
cTnT	Cardiac troponin
CTD	Cyclophosphamide, thalidomide and dexamethasone

CyBorD	Cyclophosphosphamide, bortezomib and dexamethasone
DNA	Deoxyribonucleic acid
DUAL	Doxycyline to upgrade organ response in AL amyloidosis
dFLC	Difference between involved and uninvolved free light chains
EBMT	European Group for Blood and Marrow Transplantation
ECG	Electrocardiogram
ECOG	Eastern Co-operative Group
ECV	Extracellular volume
EDTA	Ethylenediaminetetraacetic acid
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
ESRF	End stage renal failure
FLC	Free light chain
FMF	Familial Mediterranean fever

GI	Gastro-intestinal
Hg	Mercury
HR	Hazard ratio
HR	Haematological response
hsTNT	High sensitivity troponin T
ICD	Implantable cardioverter defibrillator
IF	Immunofixation
iFLC	Involved free light chain
Ig	Immunoglobulin
IL-1	Interleukin-1
IL-6	Interleukin-6
ImiD	Immunomodulatory drug
IMWG	International myeloma working group
ISS	International amyloidosis consensus criteria
ITT	Intention to treat
IVS	Left ventricular septal thickness
LC-MS	Laser capture mass spectrometry
LGE	Late gadolinium enhancement

MALDI-TOF	Matrix assisted laser desorption/ionisation
MGUS	Monoclonal gammopathy of undetermined significance
MiRAMM	monoclonal immunoglobulin Rapid Accurate Molecular Mass
Mm	Millimetre
MOLLI	Modified Look-Locker inversion recovery
MRD	Minimal residual disease
MS	Mass spectrometry
MVK	Mevolonate kinase deficiency
N	Number
NAC	UK National Amyloidosis Centre
NCBI	National Center for Biotechnology Information
NG	Nasogastric
NGS	Next generation sequencing
NICE	National Institute for Clinical Excellence

NR	No response
NS	Nervous system
NT-proBNP	N terminal pro brain natriuretic peptide
NYHA	New York Heart Association Classification
OS	Overall survival
PAD	Bortezomib, doxorubicin, dexamethasone
PBS	Phosphate buffered saline
PCN	Plasma cell neoplasm
PCR	Polymerase chain reaction
PD	Progressive disease
PEG	Percutaneous endoscopic gastrostomy
PFS	Progression free survival
PI	Proteasome inhibitor
PNS	Peripheral nervous system
pp	Phosphorylation
PR	Partial response
PSIR	Phase-sensitive inversion recovery
PTLD	Post-transplant lymphoproliferative disorder
RA	Rheumatoid arthritis

RNA	Ribonucleic acid
ROC	Receiver operator curve
RT-PCR	Reverse transcriptase PCR
SAA	Serum amyloid A protein
SAP	Serum amyloid P component
SFLC	Serum free light chain
SPE	Serum protein electrophoresis
SPECT-CT	Single-photon emission computed tomography
SSPE	Steady state free precession
99mTc DPD	99mTc-3, 3-diphosphono-1, 2-propanodicarboxylic acid
TCEP	Tris (2-carboxyethyl) phosphine
TNFα	Tumour necrosis factor α
TNT	Troponin T
TRAPS	Tumour Necrosis Factor Receptor Associated Periodic Syndrome
TRM	Treatment related mortality
TTNT	Time to next treatment
TTR	Transthyretin
UCL	University College London
ULN	Upper limit of normal
VCD	Bortezomib, cyclophosphamide, dexamethasone

VCP	Bortezomib, cyclophosphamide, prednisolone
VCTD	Bortezomib, cyclophosphamide, thalidomide, dexamethasone
VD	Bortezomib and dexamethasone
VMTP	Bortezomib, melphalan, thalidomide, dexamethasone
VRD	Bortezomib, lenalidomide, dexamethasone
VGPR	Very good partial response
wtATTR	Wild type transthyretin cardiac amyloidosis

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Chapter One

Introduction

This chapter is in the context of my publication: chapter 123: Amyloidosis**.** Helen J. Lachmann and Faye A. Sharpley. 11th Edition of Kelley & Firestein's Textbook of Rheumatology.

1.1 Overview of amyloidosis including pathogenesis

Amyloidosis is a disorder of protein misfolding, resulting in the extracellular deposition of normally soluble proteins as amyloid fibrils within the tissues, resulting in progressive organ failure. A heterogenous group of conditions can result in amyloidosis, however the amyloid deposits in all cases share a similar structure. Rigid ~10nm fibrils form a stable β pleated sheet configuration that can be visualised by Congo red staining of the tissues with the classic finding of apple-green bi-refrigence under cross-polarised light.(4) There are also a number of constant non-fibrillary components in all types of amyloid deposits including glycoprotein serum amyloid P component (SAP), apolipoprotein E and A4, laminin and Collagen IV, however the precursor protein forming the amyloid fibrils varies with more than 30 different precursor proteins described(5). There are, however, only a limited number of proteins which are able to form amyloid fibrils in vivo all of which exist in a β-structure in their normal folded state (such as β-2 microglobulin, immunoglobulin light chains and transthyretin) or are able to undergo α-helix conformation to a β

sheet formation such as apolipoproteins including serum amyloid A protein (SAA). (6) The likelihood of these native protein forming a pathological aggregate is also affected by the concentration of the protein (such as in chronic inflammatory conditions and AA amyloidosis), the protein's intrinsic tendency to assume a pathological conformation (for example in senile systemic, or ATTR, amyloidosis), or the presence of a mutation which results in a pathological form of a protein (such as in hereditary fibrinogen α chain, or Afib). The amyloid fibrils deposit within the tissues and the underlying fibril protein influence the pattern and distribution of the amyloid deposits within the organs, for example fibrinogen αchain predominately aggregates within the kidneys and β2-microglobulin in the joints. In light chain (AL) amyloidosis the light chain variable chain genes and also plasma cell burden may influence tissue tropism.(7)

1.2 Types of amyloidosis

Amyloidosis can be classified based upon the underlying cause for the protein production (hereditary or acquired), by the underlying precursor protein and also by the extent of amyloid deposition (localised or acquired) (see table 1).

Table 1.1: The types of amyloidosis and their classification

Amyloid type	Abbreviation	Precursor protein	Hereditary of Acquired	Systemic or localised
Immunoglobulin light chains	AL	Monoclonal immunoglobulin light chains	A	S or L
Immunoglobulin heavy chain (AH)	AH	Monoclonal immunoglobulin heavy chains	A	S or L
β2-microglobulin	Aβ2M	β2-macroglobulin, wild type	A	L
Systemic amyloid A amyloidosis	AA	Serum amyloid A	A	S
Wild type transthyretin amyloidosis	wtATTR	Transthyretin, wild type	A	S
Transthyretin amyloidosis	ATTR	Transthyretin, variant (due to a mutation)	H	S
Fibrinogen Amyloidosis	AFib	Fibrinogen chain, variant	H	S
Apolipoprotein A-I	AApoAI	Apolipoprotein A-I, variants	H	S
Apolipoprotein A-II	AApoAII	Apolipoprotein A-II, variants	H	S
Lysozyme	ALys	Lysozyme, variant	H	S
Gelsolin	AGel	Gelosolin, variant	H	S
Cystatin	ACys	Cystatin C, variant	H	S
A-Leukocyte	ALect2	Leukocyte chemotactic factor 2	H	S

This thesis will focus on immunoglobulin light chain (AL) amyloidosis, however, the other important sub-types of amyloidosis to be aware of as a haematologist are AA amyloidosis, hereditary amyloidosis, ATTR amyloidosis (previously senile systemic amyloidosis) and localised AL amyloidosis. These sub-categories of amyloidosis are important differentials to AL amyloidosis and will be briefly outlined

below as I touch upon these disorders in chapter nine of this thesis where I analyse the risk of developing amyloidosis post a solid organ transplant.

1.2.1 AA amyloidosis

Reactive systemic amyloidosis (AA) occurs as a consequence of chronic inflammation and most patients present with proteinuric renal disease. In AA amyloidosis the amyloid precursor protein is serum amyloid A protein (SAA), which is a normal serum component. SAA can act as an opsonin promoting bacteria uptake by neutrophils(8) and can also down regulate the inhibitory action of dendritic cells to sustain the inflammatory response.(9) This suggests that SAA, like C-reactive protein (CRP), is involved in the regulation of inflammation and immunity. SAA is synthesised predominately by hepatocytes but also by macrophages, smooth muscle cells, adipocytes and endothelial cells in response to pro-inflammatory cytokines, particularly tumour necrosis factor alpha (TNFα), interleukin-1 and 6 (IL-1 and IL-6). (10) In healthy individuals the median plasma concentration of SAA is 3 mg/ with levels exceeding 2000mg/l in an acute phase response. (11) In normal circumstances SAA is taken up by macrophages, transported to the lysosomal compartment and subsequently degraded. In patients with amyloidosis this pathway is disrupted and SAA monomers undergo proteolytic cleavage to form AA fibrils which deposit within the tissues. Glycosaminoglycan, SAP and lipid components subsequently bind to the fibrils conferring

resistance to proteolysis allowing the propagation of the characteristic β pleated sheet structure. (12)

AA amyloidosis is the third commonest type of systemic amyloidosis seen in the UK (after AL and $A\beta_2M$) and is responsible for approximately 10% of new cases seen each year. The estimated incidence of AA amyloidosis is one to two cases per million person-years. (13) This number is decreasing most likely reflecting improved treatments for chronic infection and inflammation, particularly improved access to effective anti-inflammatory medications. (14) A huge variety of underlying chronic inflammatory diseases have been reported to be complicated by AA amyloidosis (see table 2). The distribution of underlying diseases varies with geographical region and has changed over time.(14) The incidence of AA amyloidosis in patients with Familial Mediterranean fever (FMF) also varies dependent upon the geographical location of the patient and is more common in patients from Israel and Armenia, and in those with Armenian ancestry(15) with evidence of more severe FMF disease in Turkey than Germany.(16)

The median latency between onset of inflammation and diagnosis of amyloid is approximately 17 years, however the development of AA amyloidosis can occur as soon as 12 months, largely dependent on the degree of inflammation.(13) AA amyloidosis can occur at any

age and it is the major form of amyloidosis which can be seen in childhood. (17)

Any condition associated with sustained inflammation can be complicated by AA amyloidosis, including chronic infections (such as tuberculosis) and malignant neoplasms (see table 1.2). The inflammatory arthritides are the commonest cause of AA amyloidosis in the developed world, with up to 5% of patients developing the condition, however the monogenic periodic fever syndromes, such as FMF, carry the highest risk of AA amyloidosis; greater than 60% of patients with untreated FMF develop AA amyloidosis, and amyloid complicates the tumor necrosis factor receptor-1 associated periodic syndrome (TRAPS), and the cryopyrin-associated periodic syndromes (CAPS) in 25% of untreated cases.(18) As treatments for chronic inflammation are improving, there has been a suggestion that the origin of AA amyloidosis has shifted away from the inflammatory arthritides towards the rare hereditary periodic fever syndromes and conditions associated with cytokine syndromes.(8) In approximately 28% of AA patients no obvious underlying inflammatory cause can be found.(13) In such patients, an undiagnosed periodic fever syndrome or cytokine secreting Castleman's disease located in the mediastinum or gut mesentery should be considered, before the patient is labelled as having an unidentifiable cause for their AA amyloidosis.

In rheumatoid and idiopathic arthritis, the risk of developing of AA amyloidosis is linked to the duration of disease activity, (19) but not all patients with long-standing inflammation go on to develop AA amyloidosis. Likewise, in FMF development of AA amyloidosis is related to severity of disease but not all patients with suggesting that there are additional genetic, disease and environmental related risk factors.

The earliest clinical feature of AA amyloidosis is usually proteinuria. Peripheral oedema frequently prompts investigation and more than 90% of patients have non-selective proteinuria due to glomerular amyloid deposition. Haematuria rarely occurs. Nephrotic syndrome is seen in more than 50% of patients. Renal impairment is common with approximately 10% of patients presenting with end stage renal disease (ERSD).(20) The second most common presentation is organ enlargement, such as hepatosplenomegaly or thyroidomegaly. Splenic involvement is seen on SAP scintigraphy almost without exception suggesting that the spleen is the first organ to be involved, although this is usually asymptomatic. Splenic rupture is extremely rare, but can occur.(21) Approximately 10% of patients have hepatomegaly evident on examination at presentation but on SAP scintigraphy up to 23% of patients have liver involvement. (20) Elevation of serum alkaline phosphatase (ALP) is seen in 5% of patients, however this is non-specific and may not necessarily indicate hepatic amyloid deposition. Elevation of bilirubin or serum

transaminases are seldom reported and liver failure is exceptionally rare. (20) Other commonly involved organs include the gastrointestinal tract (GI) and adrenal glands. Gastrointestinal dysfunction is common in advanced disease, presenting predominantly with diarrhea and occasional bleeding.(22) Adrenal uptake is often seen on SAP scintigraphy, but few patients have adrenal insufficiency. Cardiac and neuropathic involvement are both extremely rare in AA and are features of very advanced disease.(20)

Treatment for AA amyloidosis is targeted at the underlying inflammatory condition (see table 1.2). Cytotoxic agents, such as methotrexate and azathioprine, are useful in controlling the inflammation in rheumatic disorders, such as rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis, reducing the risk of developing amyloidosis, however the majority of published evidence has focused on the biologic agents with activity against the pro-inflammatory cytokines (IL-1, TNF-alpha, IL-6). (23) There has been experience with use of the anti-IL6 receptor antibody, tocilizumab, in the treatment of AA amyloidosis with evidence for its efficacy over TNF antagonists in the reduction of SAA levels. (24) There is limited available evidence for the use of other biological agents, such as rituximab and abatacept; this may be in part due to their lack of direct anti-cytokine activity.(25, 26) Anti-cytokine therapy also has a role for the hereditary auto inflammatory conditions. Most experience to date has been with either recombinant IL-1 receptor antagonist or

monoclonal antibodies against Il-1 beta, in both attenuating the underlying disease activity and the associated amyloidosis.(27) The overall outcome for patients with AA amyloidosis has improved largely due to improved access to systemic anti-inflammatories and earlier detection. In 2007 a review of 373 cases of AA amyloidosis reported a median survival of 133 months from diagnosis (~11 years).(20) Amyloid regression is seen in patients with well controlled inflammatory levels with evidence of amyloid regression on SAP scintigraphy with a corresponding improvement in both the degree of proteinuria and renal function. The recovery of organ function is associated with excellent long-term survival outcomes.

Table 1.2: Underlying causes of AA amyloidosis and treatment

Underlying disorder	Conditions	Treatment	Examples
Inflammatory arthritis	Rheumatoid arthritis Juvenile inflammatory arthritis Ankylosing spondylitis Psoriatic arthropathy Reiter's syndrome Adult Still's disease Gout	Conventional disease-modifying agents	Gold Hydroxychloroquine Sulfasalazine Azathioprine Methotrexate
		Other immunosuppressant agents	Cyclosporine Cyclophosphamide de Mycophenolate Leflunomide
		Biologic agents	Anti TNF (e.g. Infliximab Etanercept Adalimumab) Anti-IL-6 (e.g. Tocilizumab) Anti-CD-20 Rituximab
Periodic fevers	Familial Mediterranean fever (FMF) Cryopyrin associated periodic syndrome (CAPS) TNF receptor associated periodic syndrome (TRAPS) Mevalonate Kinase deficiency (MVK)		
		Colchicine*	
		Biologic agents	Anakinra Canakinumab

			Tocilizumab
Inflammatory bowel disease	Crohns disease Ulcerative colitis	Conventional disease-modifying agents	Sulfasalazine Mesalazine Azathioprine Methotrexate
		Biologic agents	Infliximab Adalimumab
		Antibiotics	Metronidazole Ciprofloxacin Azithromycin
		Surgery	
Systemic Vasculitis	Systemic Lupus Erythematosus Polyarteritis nodosa Takayasu's arteritis Behcet's disease Systemic lupus erythematosus Giant cell arteritis/Polymyalgia rheumatic ANCA associated vasculitis	Conventional disease-modifying agents	Azathioprine Methotrexate
		Other immunosuppressant agents	Cyclophosphamide de Mycophenolate
		Biologic agents	Rituximab
		Plasma exchange	
Immunodeficiency	Hypogammaglobulinemia Cyclic neutropenia Common variable immunodeficiency Hyper immunoglobulin M syndrome	Immunoglobulins	

	Sex linked Agammaglobulinemia HIV/AIDS		
		Antibiotics	Co-trimoxazole Miconazole
Chronic Infections and conditions which predispose to infection	Chronic cutaneous ulcers Chronic pyelonephritis Chronic osteomyelitis Sub-acute bacterial endocarditis Leprosy Tuberculosis Whipples disease Cystic fibrosis* Bronchiectasis* Kartagener's syndrome Epidermolysis Bullosa IV and subcutaneous drug misuse Sickle Cell Anaemia	Antibiotics and surgery Physiotherapy*	
Neoplasia	Hodgkin's disease Renal cell carcinoma Adenocarcinoma of the lung, gut, urogenital tract Basal cell carcinoma Carcinoid Tumour Gastrointestinal Stromal Tumour Hairy cell leukaemia Hepatic adenoma Mesothelioma Castleman's disease**	Chemotherapy and surgery	
		Biologics agents	Tocilizumab**

Other conditions	Atrial myxoma SAPHO syndrome Obesity Sarcoidosis Retroperitoneal Fibrosis Inflammatory abdominal aortic aneurysm Sinus Histiocytosis with massive lymphadenopathy		

1.2.2 Hereditary amyloidosis

Hereditary systemic amyloidosis (also known as familial amyloid polyneuropathy) is an autosomal dominant inherited condition characterised by progressive peripheral and autonomic neuropathy in addition to involvement of other visceral organs, which can include the heart and kidneys. Mutations in at least nine genes can cause the condition, (28) but penetrance is variable and so it is often difficult to reliable predict the clinical phenotype based on the underlying mutation.

1.2.3 Wild-type transthyretin amyloidosis (ATTR)

Wild-type transthyretin amyloidosis (ATTR), formerly termed “senile systemic amyloidosis,” is an increasingly diagnosed cause of heart failure with preserved ejection fraction in predominately-elderly men. (29) Deposition of transthyretin most commonly occurs in the walls of the heart, but smooth and striated muscle, fat tissue, renal papillae

and alveolar walls can also be involved. The spleen and renal glomeruli are rarely affected.(30) The clinical manifestations of ATTR are due to the cardiac deposition with symptoms of heart failure, but 48% of patients have a history of carpal tunnel decompression,(31) (32), and spinal stenosis.(33)

1.2.4 Localised AL amyloidosis

In localised AL amyloidosis the amyloid deposition is confined to a single organ or site. The pathogenesis is distinct from systemic AL amyloidosis in that there is a localised growth of monoclonal plasma cells, rather than production of light chains by plasma cells within the bone marrow which suggests local production of the amyloid fibril. Localised amyloidosis can occur in almost any organ, although common sites include the skin, urinary tract (bladder, urethra and ureter) and the respiratory system (larynx and tracheal). The treatment and outcomes of localised amyloidosis are also distinct from systemic AL amyloidosis and are determined by the exact site and extent of amyloid fibril deposition.(34) Treatment is guided by patient symptoms and may involve surgical excision and laser ablation. Systemic chemotherapy usually plays no role in the treatment of localised amyloidosis. Localised amyloidosis rarely evolves to systemic amyloidosis, but patients are usually followed up by the specialist clinician for that local area (dermatologist, urologist)

who can monitor for local extension of lesions, or symptoms requiring local treatment.

1.2.5 AL amyloidosis

Systemic light chain (AL) amyloidosis is the most serious and most commonly diagnosed form of amyloidosis, accounting for 60% of new referrals to the UK centre, with an estimated incidence of five to twelve people per million person-years and a median survival of only 12 months if left untreated.(35) The condition is a complication of an underlying B-cell clone which produces immunoglobulin light chains. AL amyloidosis can complicate any of the plasma cell dyscrasias, including 15% of all cases of multiple myeloma, ~5% of all cases of monoclonal gammopathies (MGUS) and the lymphoplasmacytic lymphoproliferative disorders.(36) Compared with the plasma cell clone in multiple myeloma, translocation t (11:14) is more commonly seen and is found in almost 50% of patients, whereas gain 1q21 is seen less frequently. (37) The light chains produced by the amyloidogenic clone are unstable due to proteolytic cleavage or mutations within the variable domain affecting key structural sites. (10) The unstable light chains mis fold and aggregate which results in amyloid fibril formation. The mechanism of how the amyloid fibrils cause organ dysfunction remains unclear, but disruption of the tissue architecture and direct toxicity of the pre-fibril oligomers are thought to contribute.(11) All organs, except for the central nervous system, can

be affected by AL amyloid fibril deposition and the clinical features of AL amyloidosis depend largely upon the pattern of involvement. The most commonly affected organs are the heart (approximately 70-80%) and kidneys (approximately 60%). (12) Cardiac involvement in AL amyloidosis is the critical determinant of prognosis and causes heart failure with preserved ejection fraction. (38, 39) Renal involvement manifests with proteinuria, which may evolve into nephrotic syndrome and renal failure. The pathognomonic signs of AL amyloidosis are soft tissue involvement which includes macroglossia, periorbital purpura and submandibular thickening. Peripheral nerve involvement is seen in approximately 15% of patients and is characterised by an initial painful length dependent sensory polyneuropathy.(40) Carpal tunnel syndrome (CTS) is seen in ~20% of patients.(35) Involvement of the autonomic nervous system results in symptoms of postural hypotension, bowel disturbance or erectile dysfunction. Motor deficits can occur as a consequence of progressive peripheral nerve involvement with AL amyloidosis, or by direct amyloid infiltration of the muscles;(41) muscle involvement has been reported to carry a median survival of 12 months suggesting that myopathy in AL amyloidosis is both rare and a poor prognostic feature.(42) Arthropathy can occur in AL amyloidosis due to amyloid deposits in the synovium.(43) Patients present with a sub-acute but progressive, symmetrical arthropathy which predominately affects the shoulders, knees, wrists and small joints of the hands more than the elbows and hips. Without treatment

targeted at the underlying plasma cell clone, irreversible organ damage ensues leading to death within 12 months. (44)

1.3. Diagnosis of amyloidosis

A diagnosis of amyloidosis can be an incidental finding on biopsy of an affected organ, for example laryngeal amyloidosis on biopsy of the larynx to investigate the cause of hoarseness, or due to clinical suspicion about underlying amyloidosis, for example due to heart failure with preserved ejection fraction in a patient without a clear underling cause (see figure 1.1). In both scenarios, positive histology is the gold standard for the diagnosis of amyloidosis, with the demonstration of the presence of amyloid deposits by apple green birefringence when the tissue biopsy is stained with the aniline dye Congo red and viewed under cross polarized light (see figure 1.2) (45).

Figure 1.1: The histological appearance of amyloid deposits stained with Congo Red demonstrating apple green birefringence under cross-polarised light

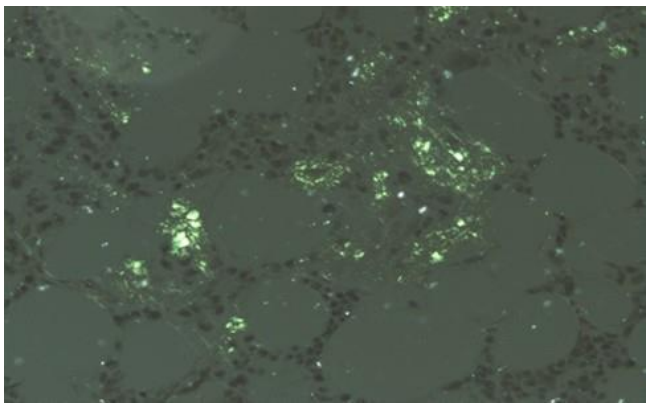
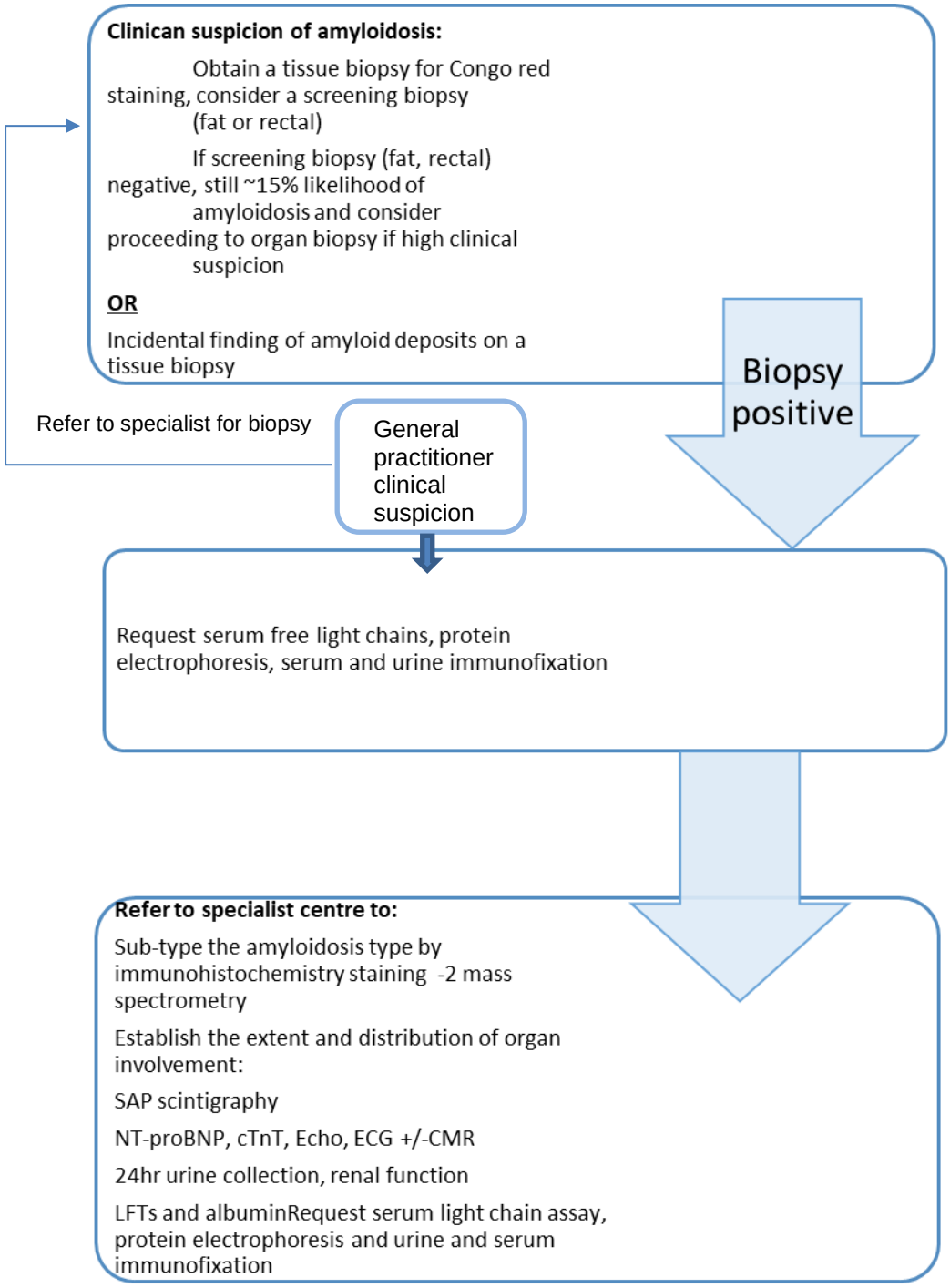


Figure 1.2: Algorithm for the diagnosis of amyloidosis and subsequent sub-typing of the amyloid fibrils.



A biopsy of the endomyocardium, liver or kidney is invasive and is associated with a risk of post procedure bleeding;(46) alternative screening biopsies include rectal, subcutaneous fat and bone marrow.(47) A negative result does not necessarily rule out amyloidosis, as amyloid deposits can be patchy. Congo red staining is also not a very sensitive test being dependent on an adequate amount of amyloid, correct staining of the tissue and adequate observer experience.(45) A diagnosis of amyloidosis is not sufficient; the amyloid deposits must be subtyped to correctly identify the underlying precursor protein and sub-categorise the type of amyloidosis which, in turn, influences treatment decisions (see figure 1.1).

In a proportion of patients high background staining makes immunohistochemistry unreliable (48) and, in such patients, mass spectrometry has a role which can identify the fibril type in over 98% of cases. (49) Genetic analysis is useful to exclude hereditary amyloidosis, especially as a family history may be absent given the incomplete penetrance. In patients presenting with a combination of autonomic and peripheral neuropathy and/or cardiomyopathy, hereditary ATTR should be excluded by sequencing of the TTR gene. Likewise, in patients presenting with isolated renal involvement should have the fibrinogen gene sequenced to exclude AFib amyloidosis.

1.4 Assessment of organ involvement

Once a diagnosis of amyloidosis has been histologically confirmed, the next diagnostic step is to assess the pattern and extent of organ involvement. In 2005, an international consensus criteria were established for the definition of organ involvement in AL amyloidosis.(50) These criteria were updated in 2010 and this forms the basis for assessing organ involvement both in clinical practice and in a research and trial setting (see table 1.3).

Table 1.3: definition of organ involvement in systemic AL amyloidosis

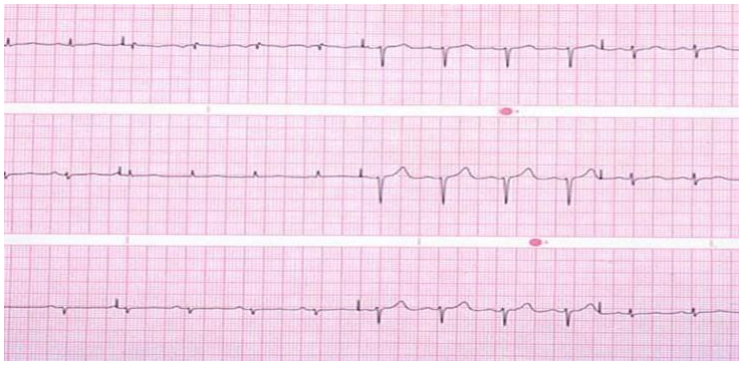
Organ Involved	**Criteria for involvement**
Heart	Echocardiogram: mean wall thickness >12mm, with no other cardiac cause
Kidney	24hr urine protein loss of >0.5g/day, predominately albumin
Liver	Total liver span >15cm in the absence of heart failure or alkaline phosphatase >1.5x the ULN
Nerve	Peripheral: clinical; symmetrical lower extremity sensorimotor peripheral neuropathy; autonomic: delayed gastric emptying disorder, pseudo-obstruction
Gastrointestinal	Direct biopsy verification with symptoms
Soft tissue	Tongue enlargement, arthopathy, claudication, myopathy by biopsy, lymph node, carpal tunnel syndrome

ULN= upper limit of normal. Adapted from Gertz (2010) A.J. Haematology

1.4.1 Diagnostic imaging

Cardiac involvement in systemic AL amyloidosis is the major factor in determining a patient's prognosis. Approximately 60% of patients will have cardiac involvement, which is assessed by a combination of defined by combination of ECG, imaging and cardiac biomarkers. The ECG may be normal, but in advanced cardiac involvement small voltages are seen and pathological 'Q' waves (pseudo-infarct pattern), (see figure 1.3). A mean left ventricular (LV) wall thickness of >12mm on two-dimensional Doppler echocardiogram, in the absence of hypertension or other causes of left ventricular hypertrophy, suggests cardiac involvement and this forms the basis for the international consensus criteria for cardiac involvement (see table 2). (50) Other features are also suggestive including diastolic dysfunction, especially when seen together with concentric LV wall thickening and impaired global longitudinal axis function.

Figure 1.3: Electrocardiogram (ECG) demonstrating small voltage complexes suggestive of advanced cardiac involvement with amyloidosis.



For patients with early cardiac involvement, establishing a diagnosis based on echocardiogram features alone can be challenging. This is also true of patients established on renal replacement therapy. Cardiac MRI (CMR) is recognised as a useful tool in such patients.

1.4.1.1 Cardiac Magnetic Resonance imaging

Cardiovascular magnetic resonance imaging has been found to have a vital role in the diagnosis and prognosis of cardiac amyloidosis.(51) Gadolinium is used as a contrast agent. In cardiac amyloidosis the extracellular space of the myocardium is hugely expanded due to amyloid fibril deposition into which gadolinium can distributed. This results in abnormal gadolinium kinetics with subendocardial late gadolinium enhancement after gadolinium contrast injection.(52) CMR is particularly valuable when echocardiography is unable to confidently define the presence of cardiac involvement due to the presence of coexisting conditions which may also lead to an increase in the left ventricular septum, such as severe hypertensive hypertrophy, hypertrophic cardiomyopathy, uremic cardiomyopathy and storage disorders. Although CMR now has a defined role in the diagnosis of cardiac amyloidosis, the role of serial CMR monitoring remains to be established. (53)

1.4.1.2 SAP (123I-SAP) scintigraphy

Serum amyloid P component (SAP) scintigraphy is an imaging method used to establish the presence, distribution and extent of amyloid deposits within the visceral organs.(54) SAP is a non-fibrillary component of all amyloid deposits and so the scan is useful for all types of amyloidosis. Radiolabeled SAP (123I-SAP) is intravenously injected and reversibly binds to amyloid deposits in proportion to the quantity present. The specific methodology is outlined in chapter 2, section 2.9.2. The dose of radioactivity is small (80-90MBq for a six-hour scan, 120-190MBq for a 24hour scan) allowing serial scans to be performed which can monitor the progression or regression of amyloid deposits over time. The scan is useful in detecting deposits within the liver, spleen, adrenal glands, bones and kidneys (see figure 1.4) (except when patients reach end stage renal failure when the lack of blood flow reduces the uptake of tracer). The resolution of SAP scintigraphy is insufficient to reliably identify deposits within the GI tract, skin, nerves, heart and lungs are also not reliably evaluated.

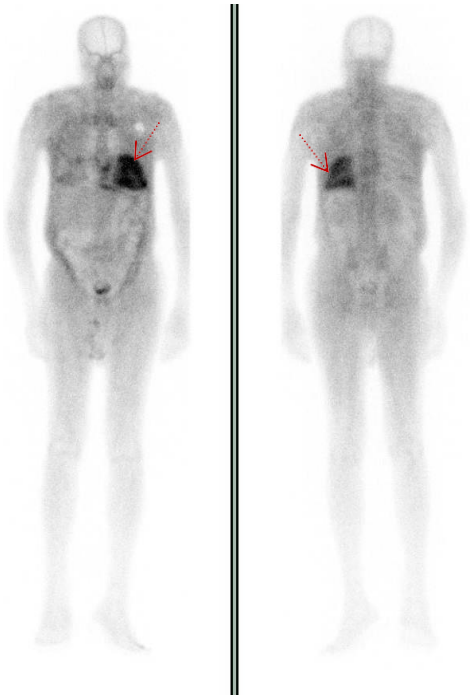
Figure 1.4: 123I-serum amyloid protein (SAP) scintigraphy scan in a patient with AA amyloidosis demonstrating amyloid deposits within the kidneys and spleen

1.4.1.3 ^{99m}Tc-DPD bone tracer scanning

Random myocardial uptake was noticed on routine bone scans during the 1970s and 1980s which were later confirmed to be cardiac amyloidosis.(55) The exact mechanism of myocardial accumulation of bone-seeking tracers in cardiac amyloidosis is unclear. Although a number of tracers have been explored, the tracer most widely used within the UK is ^{99m}Tc-DPD which demonstrates high sensitivity and

specificity of for cardiac transthyretin amyloid deposits (Figure 1.5). The specific methodology details are outlined in chapter 2, section 2.9.1. The tracer is also taken up in cardiac AL amyloidosis but the uptake is generally low grade (Perugini grade 1) in contrast to the avid high grade uptake (Perugini grade 2 or 3) in ATTR which is a helpful tool to help distinguish ATTR from AL cardiac amyloidosis(56).

Figure 1.5 ^{99m}TcDPD scintigraphy, in cardiac amyloidosis, in a patient with wtATTR cardiac amyloidosis demonstrating cardiac uptake with attenuation of the bone uptake.



1.4.2 Assessment for a clonal disorder

After histologically confirming amyloidosis and establishing organ involvement, the diagnostic work up should include serum and urine

immunofixation and serum free light chain (SFLC). The exact methods used are outlined in Chapter two. Serum electrophoresis (SPE) fails to identify the circulating M- protein in more than half of all patients with AL amyloidosis at the time of presentation. (57) Immunofixation is more sensitive, but the results are not quantitative. The SFLC assay has revolutionised the diagnosis and monitoring of AL amyloidosis. This immunoassay is highly sensitive, capable of detecting a circulating light chain excess in 98% of patients with systemic AL amyloidosis, (58) but is not specific for AL amyloidosis as monoclonal SFLCs are also found in myeloma and monoclonal gammopathy of undetermined significance (MGUS). As mentioned in chapter three, current light chain assays are also not able to distinguish normal, healthy polyclonal light chains from the pathogenic, monoclonal component, and it is here where mass spectrometry may have a role. The other limitation of SLFC assays is that absolute SFLC concentrations can increase 20–fold in renal failure. The SFLC ratio and the difference between the amyloidogenic and uninvolved SFLC concentration (dFLC) are more reliable in such cases. (59)

1.4.2.1 Bone marrow examination

A bone marrow biopsy is required to assess the plasma cell/B cell burden and determine whether the underlying clonal disorder is plasma cell or a low-grade lymphoma. The tumour burden in AL amyloidosis is usually low, with 80% of patients having a monoclonal gammopathy, with an average bone marrow plasma cell infiltration of around 7%, and only 15% of will have co-existent myeloma. (60) Half of all amyloidogenic plasma cell clones produce only light chains, with a predominance of lambda over kappa in a 4:1 ratio.(61)

1.4.2.2 Immunophenotyping and cytogenetics

Flow cytometry (MFC) can identify a clonal component and this is particularly useful in AL amyloidosis where the plasma cell component can often be subtle. Monoclonal plasma cells are detectable in 97% of patients by flow cytometry immunophenotyping. (62)

1.5 Prognostic factors

Risk stratification is an essential part of the diagnostic workup in AL amyloidosis – cardiac involvement determines the risk. In 2004, the Mayo clinic group, proposed a staging system using NT-proBNP and troponin T or I, to categorise patients with AL amyloidosis into three groups if none, one or both cardiac biomarkers are greater than the threshold levels, with increasingly poorer prognosis (median survival

of 26.4, 10.5 and 3.5 months respectively). (38) This system was re-defined in 2012 to include the dFLC.(63) NT-proBNP >8500 ng/L and systolic blood pressure <100mmHg identify a subgroup of stage III patients with a very high risk of early death.(64) Other prognostic factors and, in particular, prognostic factors to further stratify Mayo stage I patients will be outlined in chapter four.

1.6 Treatment

1.6.1 Goals of therapy

The aim of treatment in AL amyloidosis is to prevent further amyloid fibril deposition within the organs by targeting the underlying clonal plasma B cell to suppress the production of free light chains whilst supporting and preserving organ function.

Treatment also needs to be balanced against toxicity including treatment related mortality (TRM) and morbidity which is influenced by patient and disease background factors including age, co-morbidities, performance status, contraindications to drugs and patient wishes.

1.6.2 How to monitor response to treatment

Response to treatment has two components: haematologic and organ response. Consensus criteria exist for haematologic and organ responses and these are outlined in chapter two (table 2.3 and 2.4).

1.6.3 Supportive care

Supportive care is a fundamental part of treatment. As AL amyloidosis can involve almost any organ system, good supportive care requires the coordinated expertise of several specialists. Patient education is also important as patients may need to take daily weights, alter the doses of their diuretics, adhere to low salt diets. Other supportive measures include: midodrine to help with postural hypotension, albumin infusions to help patients with nephrotic syndrome, anti-diarrhoea measures including opioids (codeine phosphate) and loperamide and the addition of Octreotide in non-responsive cases. Patients with massive macroglossia or with severe GI or autonomic involvement may require nasogastric (NG), percutaneous endoscopic gastrostomy (PEG) or parental feeding. Patients with cardiac amyloidosis may also require amiodarone and cardiac monitoring during their initial chemotherapy to reduce the mortality associated with arrthymia. Implantable cardioverter defibrillators (ICD) are considered in patients with life threatening ventricular arrhythmias, but there is no definite evidence of survival advantage at present. (65) There is evidence suggesting a survival advantage for patients with cardiac stage IIIa AL amyloidosis treated with adjuvant oral doxycycline at a dose of 100mg twice a day. (66) These findings are being explored in the Doxycycline to Upgrade Organ Response in Light Chain (AL) Amyloidosis (DUAL) Trial: A Phase II Open Label Study of Oral Doxycycline Administered as an

Adjunct to Plasma Cell Directed Therapy in Light Chain (AL) Amyloidosis, which is due to complete in September 2020.

1.6.4 Systemic combination chemotherapy

The main classes of drugs with activity against AL amyloidosis are outlined in table 1.6 below.

Class of drug	Example	Mode of action
Alkylators	Melphalan Cyclophosphamide	Cross links DNA
Proteasome inhibitors	Bortezomib Ixazomib Carfilzomib	Binds 26S proteasome
Immunomodulatory (IMiD's)	Thalidomide Lenalidomide Pomalidomide	Target Cereblon, but mechanism of action not yet fully understood
Immunotherapy	Daratumumab Isatuximab Elotuzumab	Anti-CD38 SLAMF7

1.6.4.1 Alkylators and steroid regimens

Melphalan and cyclophosphamide are alkylating agents and both agents have been used, together with steroids, as the mainstay of treatment of AL for many years. The melphalan-dexamethasone (MDex) regimen, is well tolerated and associated with haematologic and organ response rates of 67% and 33% respectively. The median progression free (PFS) and overall survival (OS) are 3.8 and 5.1 years respectively. (67) The main side-effects are fluid retention and

cytopenia. There has been a move away from an alkylator-steroid doublet regimen, given the comparatively slow haematologic responses and that organ responses are rare. Approximately 5-7% of all patients with amyloidosis have an IgM secreting clone and the alkylator bendamustine is an effective treatment option for such patients. Bendamustine combined with rituximab, is an effective treatment option with an overall response rate of between 59-76%, a median PFS of 34 months and a median OS of not reached, compared with 9 months, for patients who achieved a VGPR or better compared to those who did not. (68)

1.6.4.2 Proteasome inhibitor regimes

Bortezomib is a proteasome inhibitor licensed for the treatment of multiple myeloma, AL amyloidosis and mantle cell lymphoma. The addition of bortezomib to the alkylator and steroid backbone (CyBorD or BMDex) has revolutionised the treatment of AL amyloidosis. The Mayo clinic was the first to report the benefits of this regimen in 17 patients receiving CyBorD with 71% and 24% achieving a CR and PR respectively.(69) In the UK, an initial study of 43 patients receiving CyBorD (bi-weekly Bortezomib) confirmed a haematologic response rates of 81% (CR - 42%).(70) The main side effects are peripheral sensory neuropathy, orthostatic hypotension and gastro-intestinal disturbance and the majority of AL amyloidosis patients are treated with a once weekly subcutaneous regimen. The

high response rates and good tolerability mean that CyBorD is internationally recognised first line treatment of AL amyloidosis. Ixazomib and carfilzomib are novel proteasome inhibitors with favorable toxicity profile. A retrospective analysis of 40 UK AL patients treated with Ixazomib (in combination with lenalidomide and dexamethasone) reported a median PFS of 16.6 months and a median OS of 27.3 months. Likewise, a similar retrospective study performed at the NAC of carfilzomib (combined with dexamethasone) report good tolerance and excellent responses in multiply refractory patients.

1.6.4.3 Immunomodulatory agents

The immunomodulatory (IMiD's) agents thalidomide, lenalidomide and pomalidomide are widely used in the treatment of multiple myeloma and so their use has been extended to the treatment of patients with AL amyloidosis. There are some important differences in the treatment of AL amyloidosis patients compared with the treatment of patients with other plasma cell dyscrasias. Thalidomide has unacceptable toxicity at higher doses, with up to 60% of patients experiencing grade ≥3 toxicity. (71) Despite this, CTD (cyclophosphamide / thalidomide / dexamethasone) was the first-line standard of care for patients with AL amyloidosis in the UK, until the introduction of bortezomib based regimens, with a haematological response in up to 74% of patients and a median OS of 41

months.(72)

Lenalidomide is a second generation IMiD with a more favourable toxicity profile and is used as a doublet together with steroids, or with Ixazomib and steroids for the first-line treatment of transplant ineligible patients with AL amyloidosis or those who have relapsed after first-line bortezomib based treatment. Again, like thalidomide, standard doses (lenalidomide 25 mg) are poorly tolerated, with fatigue and myelosuppression being the most common issues. (73) Patients with AL amyloidosis seem to have much better tolerance of 15 mg daily.

Pomalidomide is a third generation IMiD licensed for the treatment of relapsed multiple myeloma. The UK experience of pomalidomide is described in chapter seven. The reason for the poor tolerability of IMiD's in AL amyloidosis is largely unknown and requires further assessment. The paradoxical rise in NT-proBNP levels with treatment is also unknown and an understanding of this is particularly important given the role of NT-proBNP in assessing cardiac responses in AL amyloidosis.

1.6.5 Immunotherapy

Daratumumab is an IgG kappa monoclonal antibody targeting CD38. In 2015 Lokhorst et.al published data to prove the efficacy of

daratumumab as monotherapy in multiple myeloma.(1) In 2016 Paulumbo et.al published the findings of the CASTOR trial and since daratumumab has been widely used in combination with bortezomib and dexamethasone as second line treatment for patients with multiple myeloma. The phase 3 ANDROMEDA trial specifically looked at the use of daratumumab in combination with cyclophosphamide and dexamethasone in patients with AL amyloidosis with a haematological complete response rate of 53.3% and improved survival (hazard ratio for major organ deterioration, hematologic progression, or death, 0.58; 95% CI, 0.36 to 0.93; P = 0.02).(2) Isatuximab also targets CD38. The ICARIA-MM trial demonstrated efficacy of Isatuximab in combination with pomalidomide and dexamethasone in relapsed and refractory myeloma (3) which resulted in the NICE approval for the treatment of relapsed and refractory multiple myeloma in combination with pomalidomide and dexamethasone.(4) Elotuzumab is a humanized IgG1 monoclonal antibody targeting SLAMF7 further studies are ongoing to explore its use in patients with both multiple myeloma and AL amyloidosis (NCT03252600).(5)

1.6.6 Autologous stem cell transplantation

High dose melphalan and autologous peripheral blood stem cell transplantation (ASCT) has been used as a treatment for AL amyloidosis for over twenty years.(74) Chapter five will explore how

the toxicity of ASCT has changed over time including how appropriate patient selection has been key to this reduction in morbidity and mortality and chapter six explores whether the benefit of ASCT still holds in the era of novel therapeutic chemotherapy agents for the treatment of patients with AL amyloidosis.

1.6.7 Allogeneic stem cell transplantation

Allogeneic stem cell transplantation was first reported in 1998.(75) A transplantation (EBMT) registry study in 2005, reported a one year overall and progression free survival of 60% and 53% respectively, but with a TRM of 40% (TRM 50% in patients receiving total body irradiation).(76) This treatment approach is therefore rarely used and is reserved for highly selected, young, fit patients with relapsed disease and treatment should be at a centre with experience of transplant in AL patients.

1.6.8 Organ transplantation

The experience of organ transplantation in AL amyloidosis includes: renal, liver and cardiac transplants. Transplantation can be considered in patients who have reached end stage organ failure and who have attained a clonal response of a very good partial response (VGPR) or better. Maintenance of a clonal response is important to reduce the risk of recurrence within the graft, or progression in other organs. Renal transplantation is the most frequent organ

transplanted in AL amyloidosis. A UK series of 22 patients undergoing renal transplantation reported no graft failure secondary to amyloid recurrence and 1 and 5 year OS of 95% and 67% respectively. (77)

Liver transplantation is performed less frequently for patients with advanced liver AL amyloidosis given the overall poor outcomes. A UK study from 1984 to 2009 included 9 patients undergoing a liver transplant with a 1 and 5 year survival of 33% and 22% respectively. (77)

Cardiac transplantation accounts for 0.14% of heart transplants nationwide and is an option to improve survival in younger patients with advanced isolated heart involvement. A study of 69 patients with AL amyloidosis who were heart transplant recipients reported 1 and 5 year survival of 74.6% and 54% respectively. (78) Although this is a marked improvement over the median survival of advanced cardiac AL without cardiac transplantation scarcity of organs and the risk of amyloid recurrence within the cardiac graft still makes cardiac transplantation a contentious issue.(79)

1.6.9 Novel therapies for amyloidosis

A number of novel therapies have been developed to try and target various steps in the pathway of amyloid fibrillogenesis. R-1-[6-[R-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC) is a drug which cross-links pairs of SAP molecules in vivo

resulting in rapid clearance of SAP from the liver and almost complete depletion of plasma SAP,(80) but unfortunately this drug did not show clinical benefit and further trials have been stopped at this stage. Likewise, NEOD001 (Onclave Therapeutics Limited, California),(81) reached phase III trials but its development was halted at this stage. Prothena has taken on Birtamimab, formally NEOD001 into a phase 3 AFFIRM-AL study for patients with advanced stage disease.(6) CAEL-101 is an AL amyloid fibril reactive IgG1 antibody aimed at potentially clearing amyloid deposits with promising findings in early phase studies (7) and the drug has been taken forward to phase 3 trials at the time of writing. Future treatment options for AL amyloidosis may also include antibody-drug conjugates, bispecific T-cell engagers, and chimeric antigen receptor T cell therapy.

A number of novel drugs have been successfully developed for the treatment of hereditary ATTR amyloidosis. Tafamidis, a TTR tetramer stabiliser (82) was one of the first novel agents to hold promise for patients with hereditary ATTR amyloidosis, but an indirect comparison with Patisiran, an RNA interference therapeutic agent, suggests the superiority of Patisiran in reducing the progression of neuropathy and quality of life measures.(83) Patisiran may also improve indicators of cardiomyopathy, which is particularly exciting for patients with hereditary amyloidosis. (83) Inotersen, (84) a 2'- O-methoxyethyl- modified antisense oligonucleotide, is an

alternative and has now been NICE approved for the treatment of patients with hereditary ATTR amyloidosis with polyneuropathy. (84)

Chapter Two

Materials and Methods

2.1 Declaration

I have designed the studies described in this thesis, with the guidance of my supervisors Professor Wechalekar and Dr. Lachmann in my role as a clinical research fellow at the National Amyloidosis Centre. This thesis comprises seven studies, two of which were collaborative studies. Chapter three describes mass spectrometry as a technique to detect low levels of monoclonal serum free light chains and was supported by The Binding Site Group, Birmingham. Chapter seven describes the outcomes associated with pomalidomide in relapsed AL amyloidosis and my data from the UK cohort was used to form an international collaborative series with the Amyloidosis centres in Pavia, Italy.

The diagnostic methods described in this thesis were performed by other individuals as follows:

National Amyloidosis Centre:

- Frozen serum blood samples for the mass spectrometry project were collected by Wendy Taylor and Lois Cook and sent to The Binding Site group for analysis.

- Histological and immunohistochemical analyses were performed by Janet Gilbertson.
- Gene sequencing was performed by Dorota Rowczenio and Hadija Trojer.
- Echocardiography was performed by Babita Pawarova and Sevda Ozer.
- ^{123}I-SAP scintigraphy and ^{99m}TcDPD scintigraphy was performed by David Hutt.
- Bone marrow biopsies were carried out by clinicians at the patients' local centre, or the clinical fellows at the National Amyloidosis Centre.

Royal Free Hospital and UCLH:

- Royal Free Hospital laboratory services carried out the serum and urine biochemical and virology investigations described in chapter eight.
- Statistical support was provided by Aviva Petrie for the analyses described in chapters five and six.

2.2 Patient selection

All of the patient's described in this thesis have attended the National Amyloidosis Centre, London. At first attendance explicit written consent is obtained from all patients, for their data to be used for the

purposes of clinical research, in accordance with the Declaration of Helsinki.

All patients had a systematic review at presentation and detailed follow up assessments at six monthly intervals, or as clinically indicated. Assessment included clinical examination, detailed blood and urine analysis (including assessment of serum and urine monoclonal immunoglobulin and serum free light chains), serial ^{123}I labelled SAP scintigraphy to assess whole body amyloid load, ECG and echocardiogram. All patient data was retrieved from an electronic database, which captures all clinical data about a patient's visit to the NAC. The reported dates of death are according to the Office of National Statistics. The cause of death is reported from family members and local clinicians involved in the patient's care.

2.3 Functional assessment

A function assessment of patients is made at baseline and follow-up, based on their performance status, heart failure symptoms and ability perform a six minute walk test. Performance status was measured according to the Eastern Cooperative Oncology Group (ECOG) criteria (Table 2.1).(85) Heart failure symptoms were assessed using the New York heart association functional classification (NYHA) (Table 2.2).

Table 2. 1 Eastern Cooperative Oncology Group performance status classification(85)

Grade	Description
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 more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair

Table 2.2 New York heart association functional classification

NYHA Class	Summary	Description
I	Normal	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, shortness of breath
II	Mild	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath)
III	Moderate	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or shortness of breath
IV	Severe	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases

2.4 Haematological assessment: protein electrophoresis, immunofixation and serum free light chain assay

All patients had total immunoglobulin levels measured on a BN^{TM}II System nephelometer (Siemens, Germany). Serum protein electrophoresis (SPE) and immunofixation (IFE) (Sebia, France) were carried out using standard laboratory procedures. All patients had kappa and lambda concentrations measured at presentation using latex-enhanced immunoassay - (The Binding Site, Birmingham, United Kingdom) on a Behring BNII auto-analyser (Dade Behring, Marburg, Germany). Serial measurement of SFLC were also carried out at monthly intervals during treatment with chemotherapy. With this method the reference range is: kappa 3.3-19.4mg/L, lambda 5.7-26.3mg/L and a kappa/lambda ration of 0.26-1.65 with a lower limit of detection was <5mg/l. SFLC values were considered evaluable for assessing response if the pre-treatment dFLC was >50mg/L with an abnormal SFLC ratio. Haematological responses were assessed as per the consensus criteria (86) and are outlined in Table 2.3.

Table 2.3 - Haematological Response Criteria (86)

Clonal response	**Criteria**
Complete response	Serum and urine negative for a monoclonal protein by immunofixation Free light chain ratio normal Normalisation of both light chain classes, unless there is renal failure causing polyclonal retention of free light chain, in which case the ratio alone was used
Very good partial response (VGPR)	dFLC < 40mg/L
Partial response (PR)	If free light chain >10mg/dL (100mg/L) a 50% reduction If serum M component >0.5g/dL, a 50% reduction If light chain in the urine with a visible peak and >100mg/day a 50% reduction
Non responder (NR)	Patients who could not be classed as achieving SFLC-PR or better
Progression (PD)	From CR, any detectable monoclonal protein or abnormal free light chain ratio. From PR or stable response, free light chain increase of 50% to >10mg/dL or a 50% increase in serum M protein to >0.5g/dL or a 50% increase in urine M protein to >200 mg/day.

Table 2.4 Definition of organ involvement and organ response criteria

Organ	**Definition of Organ Involvement**	**Definition of Organ Response**
Heart	Echocardiogram: Mean wall thickness >12mm or CMR showing late gadolinium enhancement	Mean IVS decreased by 2mm, 20% improvement in ejection fraction (EF), improvement by 2 NYHA classes without an increase in diuretic use and no increase in wall thickness
Kidneys	24 hour non Bence Jones Proteinuria >0.5g, or uptake on SAP scintigraphy	50% reduction in proteinuria (at least 0.5g/day) creatinine and creatinine clearance must not worsen by 25% over baseline
Liver	SAP scintigraphy	50% decrease in abnormal ALP or reduced organ uptake on SAP scintigraphy

Spleen	SAP scintigraphy	Reduced organ uptake on SAP scintigraphy
Adrenal	SAP scintigraphy	Reduced organ uptake on SAP scintigraphy
Soft Tissue	Tongue hypertrophy, periorbital bruising, spontaneous bruising, pseudo hypertrophy, lymphadenopathy, carpal tunnel syndrome	Clinical assessment of improvement
Gastrointestinal Tract	Biopsy/verification with symptoms	
Lung	Direct biopsy verification with symptoms, interstitial radiographic pattern	Radiographic evidence of improvement in pulmonary interstitial amyloid (rare)
Peripheral Neuropathy	Symmetrical sensorimotor peripheral neuropathy in the lower limbs	Clinical assessment
Autonomic Neuropathy	Impotence, diarrhoea or constipation, early satiety and/or impaired bladder emptying without other overt cause. Orthostatic hypotension (>20mmHg fall in systolic BP)	Clinical Assessment

mm – millimetres; CMR – cardiac MRI; g – grams; SAP – Serum Amyloid P; Hg – mercury; BP – blood pressure

2.5 Bone marrow

All patients with AL amyloidosis had a bone marrow biopsy to assess the plasma cell burden. This assessment is made on the bone marrow trephine biopsy (BMT) after staining with haematoxylin-eosin stain and CD138 by immunohistochemistry. Patients with ≥10% plasma cells on BMT were classified as having AL-multiple myeloma (AL- MM) and those with <10% plasma cells as having AL-MGUS.

2.6 Histology

2.6.1 Congo red staining

The Congo red method described by Puchtler et.al (87) is used for all samples received by the National Amyloidosis Centre, including all patient samples described in this thesis. The biopsies are sent to the centre as formalin fixed de-paraffinised sections and are rehydrated and stained with haematoxylin. Congo red staining is then used and the sections dehydrated with xylene before mounting with DPX medium. Cross polarised light is then used to view the slides.

2.6.2 Immunohistochemistry

Immunohistochemistry staining was used to subtype the amyloid using a panel of anti-human monospecific antibodies reactive with: SAA (Eurodiagnostica, Huntington UK) AL kappa, lambda, transthyretin and lysozyme (Dako Ltd, Denmark House Ely UK). Apolipoprotein AI (Genzyme Diagnostics) and fibrinogen Aα chain (Calbiochem) were used where appropriate. The process involves washing formalin fixed de-paraffinised 2µm sections of tissue were then incubated in aqueous (0.3%) hydrogen peroxide (H_2O_2) for approximately 30 minutes. A rinse step follows with phosphate-buffered saline (PBS) containing 0.05% Tween (Calbiochem) solution. Non-specific tissue binding was abolished by incubation for a further 30 minutes in normal non-immune serum (Vector Part of the ImmPRESS Kit). Sections were then

incubated overnight with primary antisera at 4^{O}C, followed by a second rinse step with PBS containing 0.05% Tween (Calbiochem) and labelled with secondary antibodies. Each section was washed in PBS and bound enzyme-antibody bound complexes were visualised using a metal-enhanced DAB (Fisher Scientific solution).

2.6.2 Mass spectrometry

Mass spectrometry (MS) techniques were used to confirm the subtype of amyloidosis, where immunohistochemistry failed. For samples processed at the National Amyloidosis Centre sections of 10µm formalin-fixed, paraffin embedded tissues were cut from formalin fixed tissue on the Director *Expression Pathology 50001-024.* The Zeis Palm Micro beam Laser capture microscope was used to locate and visualise the amyloid by yellow on a “red” background. These areas were excised using laser capturing in and then incubated with trypsin and stored at -80°C (>for 1 month). Further steps of reconstitution, centrifugation, heating and cooling follow before the residues were reconstituted, vortexed and centrifuged before the supernatants were transferred for laser capture mass spectrometry (LC-MS) on the Velos. A control sample (six protein mix tryptic digest) was run before and after the samples. The data was processed using Proteome Discoverer for MASCOT and the National Center for Biotechnology Information (NCBI) database were searched to produce the mass

spectrometry results. Proteomic analysis was performed on the Velos platform and analysed following the method of Rodriguez *et al*. MS data files were analysed using Mascot software. (Matrix Science, London, UK). Details of the novel matrix-assisted laser desorption/ionisation (MALDI-TOF) MS technique used by the Binding Site group are outlined in chapter three.

2.7 Genetic sequencing

Patients with suspected hereditary amyloidosis had genotyping performed. Whole blood was stored in an EDTA tube, frozen and stored with genomic DNA isolated as required. The polymerase chain reaction (PCR) was used to amplify the coding regions for the genes and appropriate exons including: transthyretin (exons 2, 3 and 4), apolipoprotein AI (exons 3 and 4) and fibrinogen A α-chain (exon 5) using Sanger sequencing.

HotStar Taq DNA Polymerase kit (Qiagen) was used for the lysozyme gene (exon 2).

2.8 Assessment for cardiac involvement

2.8.1 Electrocardiogram

Electrocardiogram (ECG) was performed at baseline in all patients and repeated in those patients with known cardiac involvement. This was to assess for low voltage complexes, defined by a mean QRS

amplitude less than 0.5mV in leads I, II, III, aVL and aVF (as seen in figure 1.3)

2.8.2 Echocardiogram

Echocardiogram was performed on GE Healthcare: Vivid E9 (S/N VE94922, VE94921) and Vivid E9 (S/N 4544VS6). The left ventricular thickness was measured, in addition to left ventricular systolic and diastolic function and atrial diameter, all measured according to the British Society of Echocardiogram guidelines. Intra-observer variability was reduced by re-examination of all echocardiograms by a single operator.

2.8.3 Cardiac magnetic imaging

Cardiac magnetic resonance imaging (CMR) has been used to assess all patients at baseline for cardiac involvement with amyloidosis since 2015. All participants described in this thesis underwent standard CMR on a 1.5T clinical scanner (Magnetom Aera, Siemens Healthcare, Erlangen, Germany). Scans were performed in accordance with local protocol and included localizers, cine imaging (with steady state free precession (SSFP) sequence), native T1 mapping, T2 mapping, late gadolinium enhancement (LGE) imaging with phase sensitive inversion recovery (PSIR) and extracellular volume (ECV) mapping. The gadolinium-based contrast

agent used was 0.1 mmol/kg of gadoterate meglumine (Dotarem, Guerbet S.A., France).

LGE imaging was acquired using magnitude and phase-sensitive inversion recovery reconstruction (PSIR) in all patients. For native T1, T2 and post-contrast T1 mapping, 4-chamber long-axis and basal, mid-ventricular and apical short axis images were acquired using the modified look-locker inversion recovery (MOLLI) sequence for T1 after regional shimming. After a bolus of contrast and standard LGE imaging, the T1 measurement was repeated with the MOLLI sequence and ECV was calculated.

2.9 Other imaging methods

The additional imaging methods described and used for the studies described in this thesis includes: Tc-DPD scintigraphy and ^{123}I SAP scintigraphy.

2.9.1 Tc-DPD scintigraphy

Patients are injected with 700MBq of ^{99m}Tc-DPD and then scanned 3 hours post-injection using two General Electric Medical Systems hybrid SPECT-CT (single photon emission computed tomography with a low-dose, non-contrast CT scan) gamma cameras (Infinia Hawkeye 4 and Discovery 670). Whole body planar images were acquired followed by cardiac SPECT-CT. SPECT-CT reconstruction and image fusion were performed on the GE Xeleris workstation. Cardiac retention of ^{99m}Tc-DPD was visually scored using a modification of the grading devised by Perugini *et al*.(88) Grade 0 - no visible myocardial uptake in both the delayed planar or cardiac SPECT-CT scan, grade 1 - cardiac uptake on SPECT-CT only or cardiac uptake of less intensity than the accompanying normal bone distribution; grade 2 – moderate cardiac uptake with some attenuation of bone signal; and grade 3 – strong cardiac uptake with little or no bone uptake.

2.9.2 ^{123}I SAP scintigraphy

This procedure is used to visually quantify the amyloid load within the visceral organs. Patients are injected with 200µg of SAP with 190MBq of 123I. A dose of 60mg of potassium iodide is given prior to ^{123}I SAP scintigraphy and a further 5 doses administered following the scan over the following 3 days to prevent thyroid uptake. Patients have anterior, posterior and oblique imaging using an IGE-Starcam gamma-camera (IGE Medical Systems, Slough, UK) at 6 or 24 hours following the injection. A normal scan is defined by no abnormal tracer. The quantification of the amyloid load is as follows: small – uptake within one or more organs visible but with normal blood pool intensity; moderate – abnormal uptake within the organs with a diminished blood pool diminished and large – when the blood pool signal was lost. Progression of amyloid, by SAP scintigraphy, is defined as an increase of the tracer within the affected organ(s) and/or decrease in the background blood pool. Regression is defined as reduction of the tracer within the affected organ(s) and/or increase in the background blood pool.

2.10 Assessment criteria used to assess amyloid organ involvement and response to treatment

The definition of organ involvement and organ responses were both defined according to the consensus criteria and are outlined in chapter one (Table 1.3) and in this chapter, (Table 2.4). Muchtar et.al have explored the use of a graded cardiac response criteria grouping patients into: complete (nadir NT-proBNP≤350 pg/mL or BNP≤80 pg/mL); very good partial response (>60% reduction in NT-proBNP/BNP), and no response (≤30% reduction in proBNP/BNP). This is not yet used in practice and will not be mentioned in the rest of this thesis.(8)

2.11 Statistical analysis and publication

Statistical analysis was performed using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp).and Stata (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). A significance level of 0.05 was used for all hypothesis tests. The specific statistical analyses used is described in the methods section of each chapter. Approval for analysis and publication was obtained from the institutional review board at the University College London.

Results section one: advances in the diagnostic investigations and prognostication of patients with AL amyloidosis

Chapter Three

Novel mass spectrometry method detects monoclonal light chains and infers organ involvement in light chain amyloidosis

This chapter is written in the context of my publication: A novel mass spectrometry method to identify the serum monoclonal light chain component in systemic light chain amyloidosis**.** Sharpley FA, Manwani R, Mahmood S, Sachchithanantham S, Lachmann HJ, Gillmore JD, Whelan CJ, Fontana M, Hawkins PN, Wechalekar AD, *Blood Cancer Journal* **9**, 16 (2019). https://doi.org/10.1038/s41408-019-0180-1

Key Points:

- Mass spectrometry permits accurate monoclonal light chain detection against a polyclonal background in systemic light chain amyloidosis.
- The molecular light chain mass is concordant to the tissue type: ‘heavy’ in renal amyloid and a ‘light’ mass in cardiac amyloid.

3.1 Introduction

AL amyloidosis is characterised by an underlying plasma cell clone producing structurally abnormal monoclonal free light chains (FLCs) which mis-fold and deposit as amyloid fibrils leading to progressive tissue damage. Detection and serial measurements of serum FLCs are critical in determining prognosis and in assessing response to treatment. All current immunoassays for quantifying the amyloidogenic monoclonal FLCs also measure the normal polyclonal FLC background – a major limitation for a disease where even low-level monoclonal FLCs are crucially important.

Mass spectrometry (MS) is a technique used to sort a sample based on mass. MS has recently been explored in the assessment of FLCs both in the setting of AL amyloidosis and other plasma cell dyscrasias;(89) the theory being that each monoclonal FLC is made of a unique amino acid sequence, with a unique molecular mass. Various different MS techniques exist. The clonotypic peptide MS approach relies on the digestion of serum immunoglobulins with trypsin prior to analysis by MS.(90) Although this approach is sensitive,(91) the technique relies on the initial identification of a peptide from the patient's monoclonal protein (M protein)/ FLC, which can then be serially monitored over time. An alternative approach is the monoclonal immunoglobulin rapid accurate molecular mass (miRAMM) technique which, rather than analysing tryptic peptides,

utilises a reducing agent to dissociate the heavy and light chains allowing MS analysis of intact proteins. This allows both post-translational modification change to be observed (89) and minor FLC sub-clones to be monitored.(89) The matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF or MASS-FIX) is a high throughput version of miRAMM(92) which has been explored in a group of patients with plasma cell dyscrasia and has demonstrated comparable sensitivity to existing protein electrophoresis and serum FLC methods.(89)

Here we report on a novel and simple to use MALDI-TOF-MS method for monoclonal FLC detection (FLC-MS) in a series of 18 patients with systemic AL amyloidosis.

3.2 Methods

We included 18 serial patients with systemic AL amyloidosis, diagnosed and treated at the UK National Amyloidosis Centre (UK-NAC), and 2 MGUS (monoclonal gammopathy of undetermined significance) patients, referred to the NAC for ruling out a diagnosis of amyloidosis, (acting as negative controls). Two patients were selected with samples at diagnosis and post-treatment when in complete remission (CR), but with known presence of minimal residual disease (MRD) on bone marrow. Sera samples from healthy donors (n=17) were also analysed (data not shown) for comparison. A diagnosis of

AL amyloidosis was confirmed by demonstration of characteristic birefringence under cross polarized light with Congo-red staining on a tissue biopsy, and AL typing was confirmed by immunohistochemistry or by laser capture mass spectrometry. All patients had detailed baseline assessments of organ function including serum FLC measurements and imaging. Organ involvement was defined according to the international amyloidosis consensus criteria.(50)

Magnetic microparticles were covalently coated with polyclonal sheep antibodies monospecific for kappa FLCs (anti-free K) and lambda FLCs (anti-free λ). The microparticles were incubated with patient sera, washed and treated with acetic acid (5% v/v), containing tris(2-carboxyethyl)phosphine (TCEP) (20 mM), in order to elute FLCs in monomeric form. Mass spectra were acquired on a Microflex LT/SH smart matrix-assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF-MS; Bruker, GmbH). Approval for analysis and publication was obtained from the NHS institutional review board, and written consent was obtained from all patients in accordance with the Declaration of Helsinki.

3.3 Results and Discussion

Baseline characteristics of patients are presented in Table 3.1.

Table 3.1: Amyloidosis patient characteristics (n=18)

	n (%)	Median (range)
Organs involved :		2 (1-4)
Cardiac	14(77)	
Renal	8(44)	
Autonomic and soft tissue	3(17)	
Peripheral nerve	1(6)	
Liver	0(0)	
NT-proBNP, ng/L		3761 (245-25348)
Troponin T, ng/L		35 (8-170)
Serum albumin, g/L		37 (19-45)
eGFR, mL/min		62 (10-100)
Amyloid type:		
AL kappa	3(17)	
AL lambda	14(78)	
Uncertain	1(6)	
iFLC kappa, mg/L		78 (73-440)
iFLC lambda, mg/L		185 (44-1023)
dFLC, mg/L		118 (33-1015)
Monoclonal Intact Ig	14(77)	

**n=2 MGUS patients not included in table*

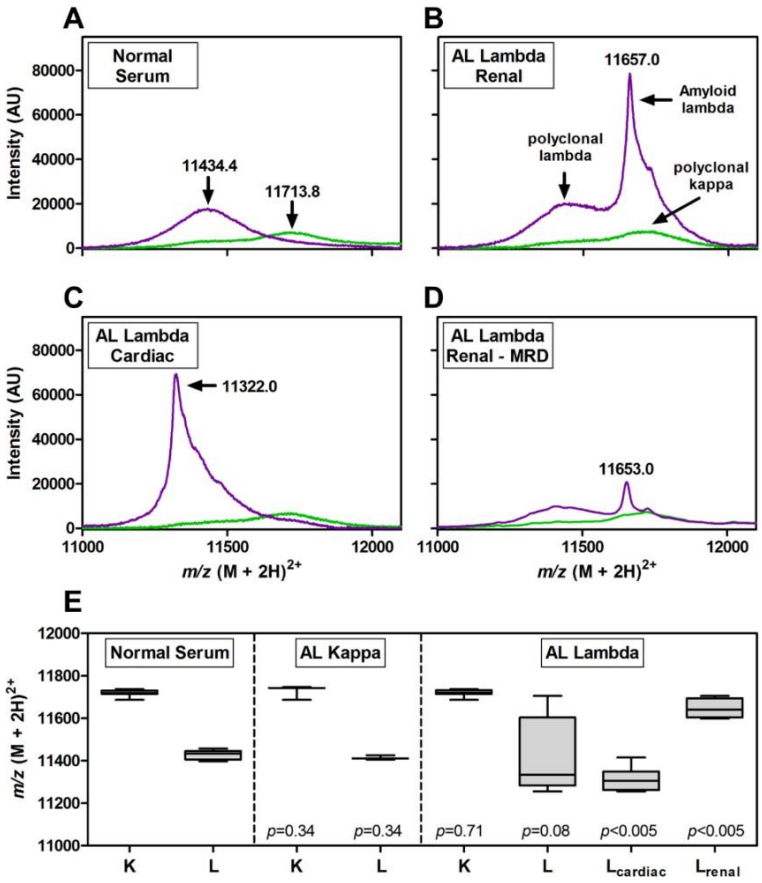
Abbreviations: NT-proBNP indicates N-terminal pro b-type natriuretic peptide; eGFR, estimated glomerular filtration rate; AL, light chain amyloidosis; iFLC, involved free light chain; dFLC, difference between involved and uninvolved free light chain; Ig, immunoglobulin

The FLC-MS assay confirmed normal polyclonal kappa and lambda FLC expression in the two control patients, and correctly identified the

presence and type of monoclonal FLC in 3/3 (100%) kappa and 14/14 (100%) lambda AL amyloidosis patients (figure 1A and B). One patient was suspected of having AL amyloidosis, but the amyloid fibril type remained unclear by immunohistochemistry and laser capture mass spectrometry; FLC-MS assay confirmed the absence of monoclonal FLC, raising a question about the diagnosis of AL amyloidosis.

Two patients had serial samples at diagnosis prior to treatment and following a serological CR. In both cases, FLC-MS identified monoclonal lambda FLC expression, (Figure 1D), with the same molecular mass at diagnosis and during CR; this was in the setting of normal FLC (lambda light chains <20mg/L in each case), and no monoclonal band in immunofixation in serum and urine. Both patients had had a bone marrow examination with next generation sequencing (NGS) and next generation flow cytometry respectively showing persistent MRD in the bone marrow to the level of <40 cells/10^6.

Figure 3.1: Mass spectra and m/z values for FLC in patients with AL amyloidosis.



In lambda AL amyloidosis patients with renal involvement, the monoclonal lambda FLC predominantly displayed a "heavy" molecular mass ($m/z^{[2+]}$ = 11646.2 ± 23.6) compared to normal polyclonal lambda ($m/z^{[2+]}$ = 11428.1 ± 4.9). Conversely, patients with cardiac involvement exhibited a monoclonal lambda FLC with a "light" mass ($m/z^{[2+]}$ = 11312.8 ± 16.1) relative to the normal control (Figure 1E). Despite the relatively low number of samples, preliminary statistical analysis (Mann-Whitney U) suggested that lambda AL amyloidosis

renal and cardiac FLCs were significantly distinct in mass from normal lambda FLCs (Figure 1E). In patients with kappa AL amyloidosis, whilst a monoclonal peak was apparent, no substantial difference in FLC molecular mass was observed when compared to normal sera (Figure 1E).

This small study describes a novel MALDI-TOF-MS technique to detect and characterise monoclonal FLC in AL amyloidosis. The Mayo group has led the way in MASS-FIX for detection of serum monoclonal immunoglobulins and FLCs, with sensitivities similar to current electrophoretic and nephelometric/turbidimetric methods.(89) The FLC-MS method described here reinforces previous findings by demonstrating: i) 100% diagnostic sensitivity and specificity; 2) 100% concordance with immunohistochemistry results; and iii) crucially identifying monoclonal light chains in patients with persistent MRD.

The unexpected and novel finding in our study is the tendency for the molecular mass to be concordant with the tissue amyloid type. Even in this small sample size, there appears to be a marked difference in FLC mass in patients with cardiac (“light” FLC) vs. renal (“heavy” FLC) involvement; although small numbers do not allow for reliable statistical analyses. This study has been extended to evaluate a larger cohort of patients but the correlation of FLC mass with tissue tropism was not replicated.(9) The pathophysiological implications remain unclear but one possibility is that as light chains are filtered by the

glomeruli, the “heavy” FLCs are trapped causing renal AL; conversely these “heavy” FLCs are not easily able to penetrate the tight cardiac capillary gap junctions, and vice versa. There is little known about tissue tropism in amyloidosis. The FLC variable region genes in AL clones and the plasma cell burden have both been associated with organ tropism.(7) However, there are reports of amyloidogenic LCs derived from the same gene demonstrating variability in their organ deposition pattern.(93) This suggests that posttranslational modifications, such as glycosylation, may be important in determining the predominance and pattern of organ involvement.

In conclusion, the unique molecular location of FLC on MS can facilitate the serial detection of amyloidogenic FLCs, allowing more accurate monitoring and more informed treatment decisions based on the monoclonal pathogenic FLC component. The ability of MALDI-TOF MS to analyse intact FLCs may be crucial in capturing post-translational modifications, which may be key in the pathogenicity of FLC in AL amyloidosis and potentially also predict organ involvement. We plan to extend the study described here to confirm our findings and to assess the impact of FLC-MS on survival and organ response outcomes.

Chapter Four

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

This chapter is written in the context of my publication: Cardiac biomarkers are prognostic in systemic light chain amyloidosis with no cardiac involvement by standard criteria. Faye A Sharpley, Marianna Fontana, Ana Martinez-Naharro, Richa Manwani, Shameem Mahmood, Sajitha Sachchithanantham, Helen J Lachmann, Julian D Gillmore, Carol J Whelan, Philip N Hawkins and Ashutosh D Wechalekar. Haematologica. 2020 May;105(5):1405-1413. doi: 10.3324/haematol.2019.217695. Epub 2019 Aug 8. PMID: 31399529; PMCID: PMC7193493.

Key points

- N-terminal pro b-type natriuretic peptide is prognostic in AL amyloidosis patients at a level much lower than the currently defined threshold (<332ng/L).
- Cardiac magnetic resonance imaging reveals cardiac involvement in a proportion of patients and is prognostic for survival.

4.1 Introduction

Systemic immunoglobulin light chain Amyloidosis (AL) is characterised by the extracellular deposition of misfolded immunoglobulin light chains resulting in progressive organ dysfunction. Patient outcomes are largely dependent upon the severity and pattern of organ involvement.(94) Accurate stratification of patients is needed to assess prognosis and to facilitate treatment decisions. Cardiac involvement is *the* critical determinant of survival. NT-proBNP (N-terminal pro b-type natriuretic peptide) is a remarkably sensitive marker of cardiac involvement and is one of the cornerstones of the international amyloidosis consensus group diagnostic criteria for cardiac involvement.(50) Change in NT-proBNP is crucial in monitoring the effect of therapy in patients with cardiac amyloidosis.(95) These findings have followed from the seminal work by the Mayo clinic group discovering NT-proBNP and TNT (troponin T) as sensitive biomarkers for prognosis in AL(38) and the development of the 2004 Mayo prognostic scoring system, which has been further refined in 2012. The widely used 2004 staging system uses thresholds of NT-proBNP <332 ng/L and a TNT <0.035 µg/l to classify patients into stage I, II or III if both biomarkers are normal, one biomarker elevated or both biomarkers elevated respectively.(38) This is with progressively poorer prognosis (median survival of 27.2, 11.1 and 4.1 months respectively). Lately, with the move to high sensitivity troponin T (hsTNT), the threshold for troponin is <0.55 ng/L.

Recent studies of patients with normal NT-proBNP and hsTNT without cardiac involvement, (so called Mayo stage 1 disease) show excellent

outcomes with median overall survival (OS) not reached at 5 years. There are still deaths in this group of patients and few have explored factors predictive of poor survival. There are a number of novel prognostic variables in AL including: number of organs involved, a high percentage of bone marrow plasma cells,(74) raised von Willebrand factor(96) and high growth differentiation factor-15 levels.(97) None of the studies have focused specifically on the stage I patients. Liver involvement is widely believed to contribute to the poor prognosis of such cases but in the vast majority of cases this is associated with other organ involvement.(98)

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

4.2 Methods

This study included all prospectively followed up patients with AL amyloidosis from an ongoing prospective observational study (Alchemy) from 2009-2017 with Mayo Stage 1 disease (defined by normal cardiac biomarkers (NT-proBNP <332 ng/L, hsTNT <55 ng/L)). A threshold of hsTNT of 55 ng/L was used since this had been previously identified as part of equivalence testing (as equivalent to the 0.035 µg/L cTNT) by our laboratory when we moved to using hs-TNT measurements from standard troponin-T measurements at our centre.

A diagnosis of amyloidosis was confirmed by Congo-red staining of a tissue biopsy with the demonstration of characteristic birefringence under cross polarized light and AL typing was confirmed by immunohistochemistry with specific antibodies or by mass spectrometry. Hereditary amyloidosis was excluded by appropriate gene sequencing as needed. As part of the study protocol, all patients had a detailed baseline assessment of organ function, including biomarker measurements and imaging with echocardiogram and ^{123}I-labelled serum amyloid P (SAP) scintigraphy. Organ involvement was defined according to the international amyloidosis consensus (ISS) criteria.(50) Specifically, the echocardiogram was considered to show cardiac involvement if the patients had mean left ventricular (LV) wall thickness >12 mm, in absence of any other cause of left ventricular hypertrophy. NT-proBNP was <335 ng/L and high sensitivity cardiac troponin T (hsTNT) <55 ng/L in all cases. Cardiac magnetic resonance imaging (CMR) was added to routine baseline assessments from late 2015 onwards and the result of the baseline CMR was recorded, where available. A typical pattern of late gadolinium enhancement and an extracellular volume (ECV) >0.30 on an MRI were used as criteria suggestive of cardiac involvement by CMR.(51)

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

renal and liver function and treatment given. Since asymptomatic liver involvement is often detected by ^{123}I -SAP scintigraphy (99) we assessed the prognostic significance of amyloid load by this imaging method. Survival outcomes were analysed using the Kaplan-Meier method with comparisons done using the log rank test. All p-values were two sided with a significance level of < 0.05 and median values were used to dichotomise continuous variables. Any factors found to be significant on univariate analysis were further assessed in multivariate modelling by Cox's regression analysis. Statistical analysis was performed using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp).and Stata (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). Approval for analysis and publication was obtained from the NHS institutional review board and written consent was obtained from all patients in accordance with the Declaration of Helsinki.

4.3 Results

A total of 378 patients were included in this study. The patient baseline characteristics are outlined in table 4.1. The median patient age was 69 years (range 35-92 years); 212 (56.1%) were men. The median number of organs involved was 2 (range 1-7). *None* of the patients had cardiac involvement by standard criteria.(86) The majority of patients had renal involvement (n=277, 73.3%). Thirty-nine patients (10.3%) had liver involvement by ISS criteria, whilst liver was abnormal

by ^{123}I-SAP scintigraphy in 111 (29.4%). By ^{123}I-SAP scintigraphy, amyloid deposition was

Table 4.1: Baseline patient characteristics, (total patients, N=378) including univariate analysis.

Factor assessed for significance	**Median (range), N(%)**	**HR (CI)**	**Cox regression P value**
Age (years), >70 years	69 (35-92), 93 (25)	1.034(1.010-1.059)	**0.005**
Male sex	212 (56.1)	0.850(0.667-1.082)	0.186
Number of organs involved	2 (1-7)		
Renal	277 (73.3)	0.804 (0.486-1.330)	0.396
PNS	43 (11.4)	1.612 (0.866-3.000)	0.132
ANS	30 (7.9)	2.177 (1.144-4.142)	**0.018**
Soft Tissue	44 (11.7)	1.792 (0.982-3.273)	0.057
GI	36 (9.5)	1.428 (0.731-2.789)	0.297
Spleen	160 (42.3)	1.279 (0.759-2.154)	0.354
Renal parameters			
Creatinine (µmol/L)	76 (27-487)	1.004 (1.000-1.008)	0.036
eGFR (ml/min)	69 (18- >90)	0.990 (0.972-1.008)	0.274
eGFR < 30ml/min	14 (3.73)	2.11 (0.262-17.047)	0.483
Proteinuria (g/24h)	4.28 (0.03-58.46)	0.99 (0.997-1.001)	0.198
Liver parameters			
Albumin (g/L)	32 (15-50)	0.994(0.968-1.020)	0.633
Bilirubin (mmol/L)	5 (1-57)	1.00(0.998-1.001)	0.630
ALP (U/L	77 (31-2112)	0.923 (0.561-1.519)	0.753
Abnormal ALP (<129U/L)	47 (22.9)	0.872(0.352-2.155)	0.766
Liver involvement (ALP 1.5x upper limit)	39 (10.3)	1.518 (0.797-2.891)	0.204
SAP liver involvement	111 (29.4)	0.750 (0.443-1.269)	0.284
SAP load None/equivocal Small/Moderate/Large	 122 (32.4) 181 (48.0) 74 (19.6)	 0.956(0.489-1.869)	0.894

Cardiac parameters			
NT-pro-BNP (ng/L)	161 (8-330)	1.006 (1.003-1.009)	**<0.001**
NT-pro-BNP >152 (ng/L)	208 (55)	2.413 (1.448-4.021)	**0.001**
hsTNT (ng/L)	10 (3-51)	1.032 (1.011-1.054)	**0.003**
hsTNT >10 (ng/L)	76 (37.1)	1.249(0.554-2.813)	**0.592**
Echocardiogram (mean LVW)	10 (6-13)	0.998(0.820-1.215)	0.984
Haematological parameters			
Presenting kappa (mg/L)	22.55 (1.5 -935)	1.101 (0.847-1.203)	0.916
Presenting lambda (mg/L)	26.6(1.9- 6180)	0.991 (0.831-1.181)	0.917
dFLC (mg/L)	1.40 (0.1-6064)	0.991 (0.831-1.181)	0.919
dFLC > 50mg/L dFLC >180mg/l	104 (28.2) 51 (13.5)	1.431 (0.859-2.384) 1.590(0.848-2.979)	0.202 0.143
Treatments			
PI based IMiD based Alkylator ASCT No treatment/ trial treatment* Missing data	248 (67.4) 164 (44.6) 43 (11.7) 55 (14.9) 24 (6.5) 10 (2.6)	0.732 (0.417-1.287) 1.560 (0.937-2.599) 1.084 (0.529-2.224) 0.476 (0.143-1.591)	0.279 0.088 0.825 0.137
Treatment interval			
2008-2012 2012-2016 2014-2016 2016-2018 No treatment/ missing data	29 (8.4) 88 (25.5) 80 (23.2) 77 (22.3) 33 (9.6)		

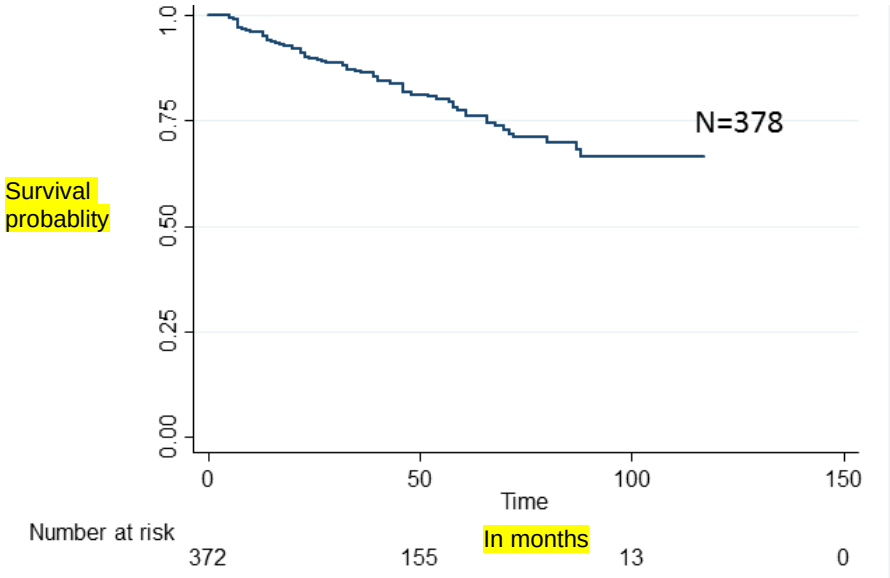
*Trial treatment MLN9708. PNS, peripheral nervous system ; ANS, autonomic nervous system ; GI, gastrointestinal; NT-pro BNP, N-terminal pro b-type natriuretic peptide; hsTNT, high-sensitive cardiac troponin T; dFLC, difference between involved and uninvolved serum free light chains; ALP, alkaline phosphatase;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

seen in 255 patients with the distribution: no amyloid in 122 patients (32.4%); 181 patients (48.0%) had a small or moderate amyloid load and 74 (19.6%) had a large amyloid load. The mean LV wall thickness was 10mm (range 6-13mm). Six patients had a mean LV

thickness of 13mm, but none with echocardiogram appearances suggestive of cardiac amyloidosis based on their preserved global strain pattern. In all six patients the NT-proBNP was <335 ng/L, and co-existing hypertension was present in 5/6. The median NT-proBNP was 161 ng/L (range 8-330 ng/L) and hsTNT was 10 ng/ml (range 3-51 ng/L). Peripheral and autonomic neuropathy were seen in 43 (11.4%) and 30 (7.9%) cases respectively.

The median follow up was 42 months (1-117 months). There were 71 deaths. Median OS was not reached (Figure 4.1A).

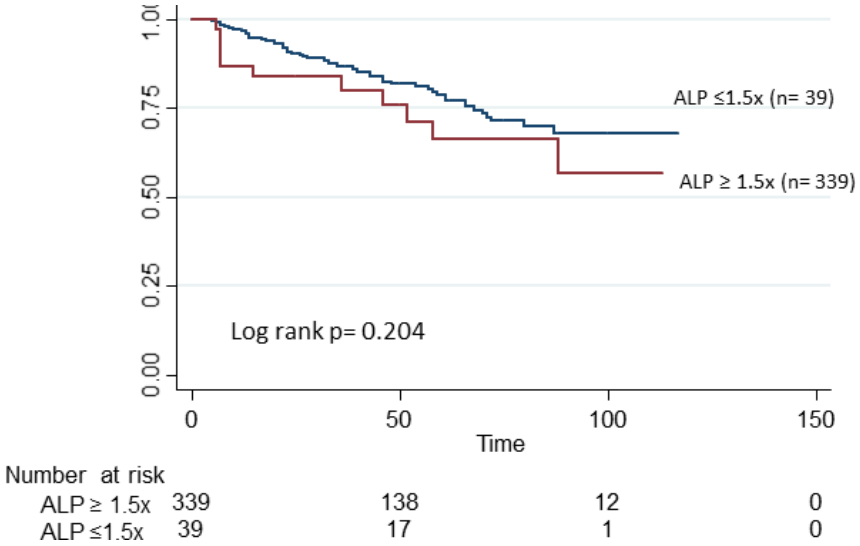
Figure 4.1 A: Survival curves for Mayo stage 1 patients demonstrating overall survival was not reached



The OS at 1, 3, and 5 years was 96%, 87% and 78% respectively. Liver involvement by ISS (ALP > 1.5 x upper limit of normal (ULN)) was not prognostic for survival (p=0.204, HR=1.518 CI=0.797-2.891), neither was any abnormality in the ALP (defined by an ALP outside the

ULN of 129U/L) (p=0.753, HR= 0.923, CI=0.561-1.519). Although liver involvement was detected more frequently on SAP scintigraphy, neither liver involvement by SAP (p=0.284, HR=0.750, CI=0.443-1.269), nor the amyloid load on SAP scans (p=0.894, HR=0.956, CI= 0.489-1.869) were prognostic for survival (Figure 4.1B)

Figure 4.1 B Liver involvement was not prognostic for survival (p=0.204, HR=1.518 CI=0.797-2.891), neither was any abnormality in the ALP (defined by an ALP outside the ULN of 129U/L) (p=0.753, HR= 0.923, CI=0.561-1.519)

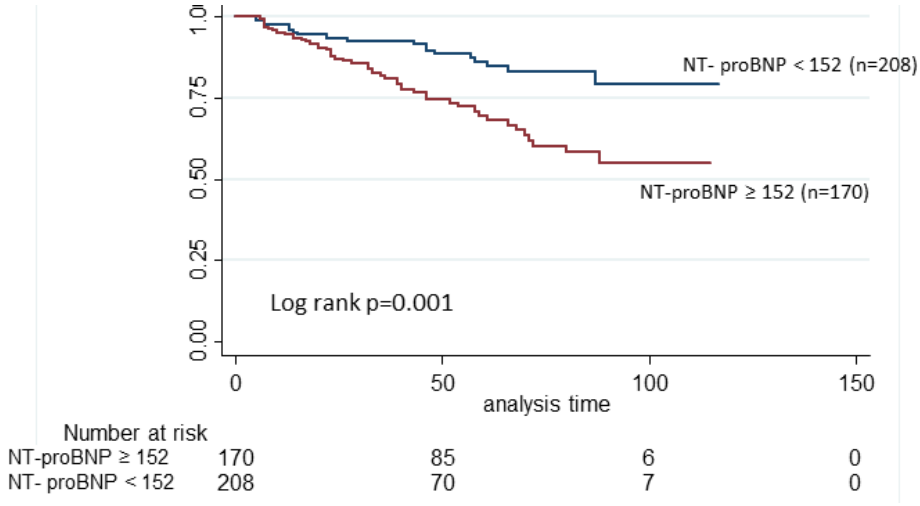


Renal involvement was not predictive of outcome using the standard consensus criteria definition, (86) (p=0.396, HR=0.804, CI=0.486-1.330), or an eGFR of <30 mls/min (p=0.483, HR=2.11 CI=0.262-17.047), but only 14 patients had an eGFR <30mls/min and only 5 patients had an eGFR <20mls/min. Patients with autonomic nervous

system involvement had significantly poorer outcomes on univariate analysis (p=0.018, HR**=**2.177, CI=1.144-4.142) but patient numbers were small. Age was predictive of survival on univariate analysis (p=0.005, HR=1.034, CI=1.010-1.059) but using receiver operating characteristic (ROC) analysis there was no clearly identifiable threshold for poorer outcomes. The presenting free light chains were not prognostic for survival in this cohort as a continuous variable or a dichotomous variable above or below a dFLC of 50mg/L or 180mg/L (table 4.1). At four years 83% versus 77% of patients with a dFLC above or below a value of 50mg/L were alive (log rank p= 0.202).

Although all the patients included in this study had no evidence of cardiac involvement and had biomarkers below the threshold for defining cardiac involvement, hsTNT and NT-proBNP were still prognostic for survival both on univariable analysis and only NT-proBNP on multivariate analysis. We undertook ROC analysis to define thresholds for NT-proBNP and hsTNT, (identified as 152 ng/L and 10 ng/L respectively), as prognostic cut offs for poorer survival. The OS was significantly better for patients with NT-proBNP <152 ng/L vs. those with a greater value (although median OS not reach for either group) (log rank p=<0.001; figure 4.1C).

Figure 4.1 C N-terminal pro b-type natriuretic peptide (NT-pro-BNP) above and below 152 ng/L showing poorer outcome for patients with NT-proBNP >152 ng/L, (log rank p= 0.001);

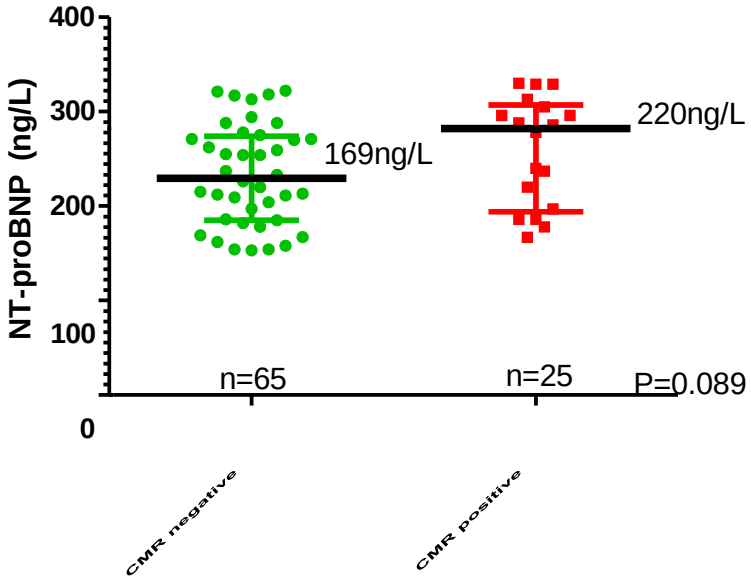


At 1, 3, and 5 years, for patients with NT-proBNP below and above 152 ng/L, the OS was 96% vs 94%; 91% vs 82%; and 83% vs 70% respectively. The OS at 1, 3, and 5 years for patients with hsTNT below and above 10 ng/L was 98%% vs 93%%, 91% vs. 84% and 87% vs 70% respectively. The median OS was not reached for either group. There was no significant difference in the median creatinine or eGFR for patients with a NT-proBNP value </ ≥ 152ng/L (p=0.091 and 0.206 respectively) ruling out impairment of renal function as a cause of abnormal NT-proBNP in this cohort.

CMR was undertaken since 2015 and results were available on 90/378 (24%) patients. Twenty-eight percent (n=25/90) of patients had cardiac involvement by CMR. In the patients who had a CMR with NT-proBNP below (32 patients) and above (58 patients) 152 ng/L, the

CMR was positive for amyloid deposition in 22% vs 31% of cases, respectively (p=0.353) (see Table 4.2). There was a trend towards higher NT-proBNP in patients with a positive CMR median NT-proBNP 220 ng/L vs. 169 ng/L (p=0.089) (Figure 4.1D).

Figure 4.1D The difference in N- terminal pro b-type natriuretic peptide (NT-pro-BNP) between patients with, and without, evidence of cardiac involvement on cardiac magnetic resonance imaging (CMR).



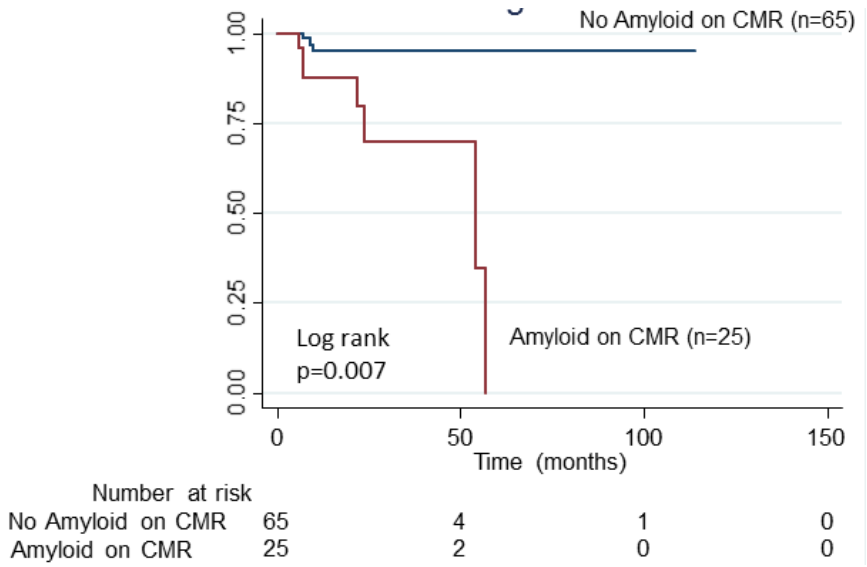
The median LV wall thickness by echocardiogram (11mm vs. 10mm (p=0.1902)) and hsTNT values (17 ng/L vs. 14 ng/L (p=0.373)) were not significantly different in those patients with CMR positivity for amyloid deposition compared to those patients with negative CMR findings.

Table 4.2: A comparison of patients with N-terminal pro b-type natriuretic peptide >152ng/L vs <152ng/L.

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

After gadolinium contrast, the extracellular volume fraction (which directly reflects myocardial interstitial expansion by amyloid deposition) was calculated with a median ECV of 0.33 (0.24-0.71). The mean ECV of patients with cardiac involvement was 0.44 vs. 0.31 ($p<0.0001$) for those without cardiac involvement. Cardiac involvement on CMR was prognostic for OS with the 1- and 2-year survival for patients with CMR positive vs. negative being 86% vs 98% and 69% vs 98% respectively (p= 0.007, HR=6.563, CI=1.689-25.492) (Figure 4.2).

Figure 4.2 Cardiac magnetic resonance imaging findings demonstrating a significantly poorer outcome for patients with cardiac amyloid deposition, (log p= 0.007)



Too few patients have sufficient follow up for meaningful longer-term survival analysis at present.

Treatment details were available in 97% of cases (N=368/378) and are outlined in table 4.1. A total of 91% (N=346/378) patients were treated with chemotherapy. The most common treatment given was Bortezomib combination regimen (mostly cyclophosphamide-bortezomib-dexamethasone) (N= 246/368, 67%) followed by thalidomide (mainly cyclophosphamide-thalidomide-dexamethasone) (N=110/369, 30%). Fifteen percent (N=55/368) of patients has an upfront autologous stem cell transplant (ASCT). Treatment type was

not prognostic for survival on univariate analysis (table 4.1).

In the 346 patients who received chemotherapy 89% (N=337/378) were evaluable at six months. Haematological response was as follows: complete response (CR) 51% (N=173/378, very good partial response (VGPR) 13% (N=46/346), partial response (PR) 3% (N=12/346), no response (NR) 4% (N=14/346) and progressive disease (PD) 17% (n=58/346). The overall survival of patients who achieved a CR to treatment was significantly longer than those who did not achieve a CR (median OS 109 vs 75 months, P <0.001), (Figure 1B). The six-month landmark analysis was as follows: CR- median survival not reached, non-CR median survival 88 months, P <0.001. Survival at one and three years by NT-proBNP <152ng/L was: CR=100%, 96% vs non-CR: 90%, 69% respectively, and for patients with NT-proBNP >152ng/l: CR= 96%, 80% and non-CR: 91%, 53% respectively, P= <0.001.

Of the 346 patients treated, 80% (N=277/346) had NT-proBNP readings at 12 months. Based on a cut-off of 30% change in NT-proBNP to define response: 32% (N=88/277) patients had reduction in their NT-proBNP levels, 50% (N=138/277) patients' values increased and 18% (N=51/277) patients did not reach either criteria. When analysing the entire cohort there was no significant difference in survival between patients who had an NT-proBNP response versus no response/ progression, (P=0.193); the 3-year survival of patients was 76% versus 70% for patients with an NT-proBNP response compared with unchanged/progression, respectively.

However, when the analysis was restricted to patients with NT-proBNP >152 ng/L, outcomes were significantly poorer in the patients with a baseline NT-proBNP level of >152ng/l who progressed (P= 0.001).

Multivariate models were developed using variables significant on univariate analysis, defined as a P value <0.05, (table 4.3). A model including CMR was done separately due to the limited number of patients with CMR data. On multivariate model including age, autonomic nervous system involvement, NT-proBNP >152 ng/L, hsTNT >10ng/L, only NT-proBNP (p=0.008, HR=3.180, CI=1.349-7.495) was an independent predictor of survival, (table 4.1). When cardiac involvement by MRI was added to the model, only cardiac amyloid on CMR (p=0.026, HR=5.360, CI=1.219-23.574) remained an independent predictor of outcome.

Table 4.3: Factors included in a multivariate analysis and their significance (separate multivariate models were developed with and without CMR due to smaller patient numbers with CMR data).

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

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

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

a proportion of patients the cause of death was progressive cardiac amyloidosis.

4.4 Discussion

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

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

importance of NT-proBNP for defining cardiac involvement is reflected in the initial Mayo staging scoring system where a threshold for NT-pro-BNP was defined using a multivariate model with a value of 332 ng/l (the upper reference limit of normal for women older than 50 years) providing the best fit and the highest hazard ratio (table 4.4). (38)

Table 4.4: A review of the literature to outline previous studies and the previous prognostic thresholds of NT-proBNP

Study details	*NT-proBNP threshold*	*Survival*
Palladini G *et. al.* 2003 *(100)*	152 pmol/L= 1288ng/L	7.6 per 100 person-years (95% CI, 3.6 to 15.7) and 72.2 per 100 person-years (95% CI, 54.2 to 86.1)
Dispenzieri A. et.al 2004 (38)	332 ng/L	<332pg/ml survival 20 months >332pg/ml 5.8 months
Kumar SK et.al 2011(101)	332 ng/L	Median OS from diagnosis for patients NT proBNP <332ng/L was 4.0 years vs 2.4 years if either NT-proBNP was >332ng/L or cTNT >0.035 µg/L.
Wechalekar AD et. al 2011 (102)	NT-proBNP <15 pmol/L= 127 ng/L	5-year survival 98% *versus* 88% for those above and below respectively
Kumar S et.al. 2012(63)	1,800 pg/mL= 1800ng/L	NT-ProBNP ≥ 1,800 pg/mL was 10.5 months, compared with median not reached for those with NT-ProBNP < 1,800 pg/mL

The prognostic importance of this value has since been confirmed in a number of studies although the threshold value itself has never been systematically re-examined. In 2011 we reported a small cohort of patients with NT-proBNP <127 ng/L had much better outcomes and those with NT-proBNP >127 ng/L had a higher risk of developing cardiac amyloidosis on longer term follow up. (102) In the 2011 cohort,

we had not access to MRI scanning understand the relevance of these findings. Dittick et.al (2019) have also highlighted the difficulty of using current Mayo staging scores in the setting of renal impairment and atrial fibrillation. (103) The Mayo Clinic data, and data from the international collaborative series, were also generated in the era where highly effective novel agent-based therapies were not routinely available. The survival of patients with stage one disease in these earlier series may now be considered relatively poor compared with contemporary survival outcomes – allowing for a potential opportunity to revisit the NT-proBNP threshold for defining cardiac involvement.

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

The exquisite prognostic sensitivity of NT-proBNP in AL amyloidosis may suggest either early cardiac involvement or light chain

proteotoxicity. The structurally established echocardiographic criteria for AL cardiac involvement is an LV wall of >12 mm, (in absence of other causes). It is conceivably possible for a patient with baseline 8-10mm LV wall could have substantial amyloid deposition before the threshold of 12mm is reached. The opportunity to track changes in NT-proBNP during development of cardiac AL is rare. The kinetics of NT-proBNP increase as well as its correlation with LV wall thickness at early stage of the disease process remain largely unknown. Dittrick et.al. have also

CMR is an alternative method of monitoring patients with cardiac amyloidosis. In this current cohort, a third of all patients who had a CMR showed features of cardiac amyloidosis. Moreover, the presence of amyloid deposition on CMR was an independent prognostic marker. CMR, with late gadolinium enhancement (LGE) and T1 mapping, is emerging as a highly sensitive and specific tool for diagnosis and characterisation of cardiac amyloidosis in AL, (Figure 5.3). (104) Transmural LGE with phase-sensitive inversion recovery (PSIR) is associated with the burden of cardiac amyloid and predicts death independent of NT-pro-BNP and other known prognostic factors. (51)

Figure 4.3: CMR image of a patient with no evidence of cardiac amyloidosis by echocardiogram and NT-BNP <332 ng/L showing characteristic features of cardiac involvement: 3A: Four-chamber steady state free precession (SSFP) cine (top right panel); 3B: corresponding native T1 map (top left panel) with an elevated value of 1209m; 3C: corresponding phase sensitive inversion recovery late gadolinium enhancement (PSIR LGE) image showing subendocardial LGE (bottom right panel); 3D: corresponding extracellular volume (ECV) map with an elevated value of 0.47 (bottom left panel)

Figure 4.3A

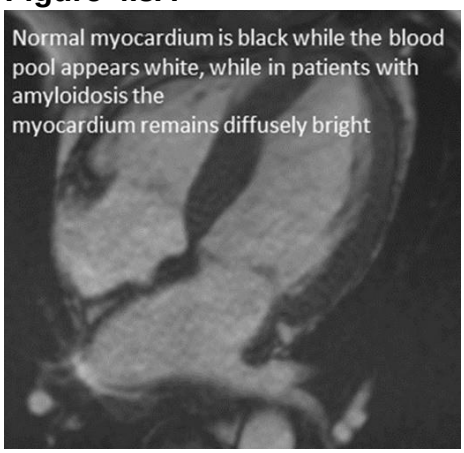
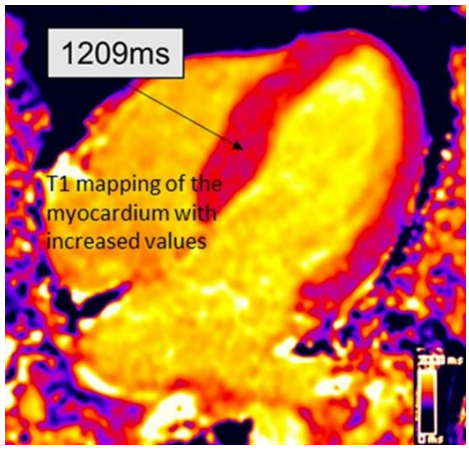


Figure 4.3B



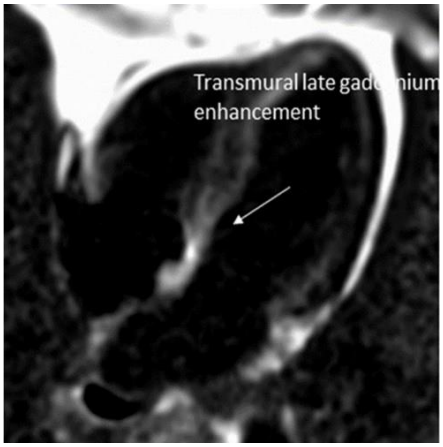


Figure 4.3C

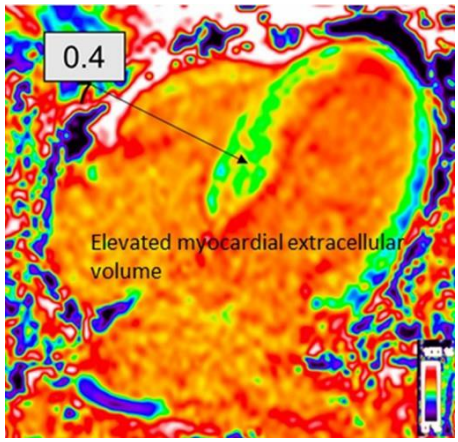


Figure 4.3D

In this cohort, it clearly identified cardiac involvement in patients where the echocardiogram was not suggestive of cardiac amyloidosis but not all patients with NT-proBNP >152 ng/L had abnormal CMR (31% had abnormal CMRs) and, conversely, 22% with NT-proBNP <152 ng/L had abnormal CMRs. This suggests that CMR provided complementary information on patients' cardiac damage. NT-proBNP may be detecting cardiac damage by light chain proteotoxicity before structural amyloid deposition is apparent on CMR, conversely, a small proportion of AL patients may have non-proteotoxic light chains (analogous to cardiac amyloid deposition in ATTR (transthyretin) amyloidosis) where the structural changes are apparent on CMR before biomarkers become abnormal. In this early stage of the disease, NT-proBNP and CMR findings should be used together for defining cardiac involvement.

In this study liver involvement, a previously reported poor prognostic marker, (98, 105) was not significant. Relatively few patients had significant liver involvement – only 10% by consensus criteria (although a third had asymptomatic liver involvement on ^{123}I-SAP scintigraphy). The strict exclusion of cardiac involvement by consensus criteria may have excluded patients with advanced liver involvement since the latter patients often have multi-organ amyloidosis. Likewise, although the majority of patients had renal involvement, 277 (73.3%) the median presenting creatinine was low (76 µmol/L), with only a small proportion (N=14/375, 3.7%) with an

eGFR < 30 mls/min, which may explain why neither the presence of renal involvement nor proteinuria was a predictor of survival. ANS involvement was significant on univariate but not multivariate analysis and the number of patients with ANS involvement was small.

This study has limitations and needs to be interpreted in this context. This is single centre data but we are planning validation in an international collaborative data set. One major limitation is that the exact cause of death was only available in a small proportion of patients with the cause of death recorded as “amyloidosis” which does not elucidate whether cardiac amyloidosis or other was the real cause of death. Progressive cardiac amyloidosis does appear to be the cause of death in at least a proportion of patients in this study, based on serial echocardiogram imaging. The use of a very sensitive marker of cardiac disease like NT-proBNP at a low level is also challenging as other unrelated factors which impact upon NT-proBNP (such as age, renal function, sex, body mass index as evidenced by the Framingham study from 2011, and a more recent study by Dittrick et al. (2019). (103, 106) Only a small number of patients had CMR scans. Larger studies are needed to address these limitations.

In conclusion, this study demonstrates that in patients with AL amyloidosis with no cardiac involvement by consensus criteria even small elevations of NT-proBNP as well as cardiac involvement by CMR are factors highly prognostic for survival. This novel finding offers

some insight into the heterogeneity in survival of Mayo Stage 1 patients. There findings have implications for clinical practice (but need to be confirmed in larger international collaborative studies). A baseline cardiac MRI scan, if available, should be considered at diagnosis for stage I AL patients. Better outcomes for patients in a CR and those with decrease in NT-proBNP, suggest that in “high risk” stage 1 patients (those with NT-proBNP >152 ng/L) the goal of therapy should be similar to those with cardiac AL i.e. a complete haematological response. The follow up of such patients should include routine NT-proBNP measurement including assessment of response (as patients with presenting NT-proBNP >152ng/L and NT-proBNP progression (>30% increase) had poorer outcomes); those with NT-proBNP progression should be considered for further treatment. The “high risk relapse criteria” defined by the Italian Amyloidosis group, should be applied for treatment at relapse for patients with NT-proBNP >152 ng/L (high risk stage I). (107) Data in serial CMR is needed to assess cardiac structure and functional changes to delineate the natural history of such ‘high risk’ patients to help identify interventions to prevent progressive cardiac involvement.

Results section two: treatment options for patients with AL amyloidosis

Chapter Five

Autologous stem cell transplantation for light chain amyloidosis

This chapter is written in the context of my publication: A twenty-four year experience of autologous stem cell transplantation for light chain amyloidosis patients in the United Kingdom. Faye A Sharpley, Aviva Petrie, Shameem Mahmood, Sajitha Sachchithanantham, Helen J Lachmann, Julian D Gillmore, Carol J Whelan, Marianna Fontana, Ana Martinez De Azcona Naharro, Cristina Quarta, Philip N Hawkins, and Ashutosh D Wechalekar Br.J.Haematol. 2019 Dec;187(5):642-652. doi: 10.1111/bjh.16143. Epub 2019 Aug 13. PMID: 31410841.

Key points:

- Autologous stem cell transplant is an increasingly safe treatment for patients with AL amyloidosis with a significant reduction in transplant related mortality over time

- UK survival outcomes for patients with AL amyloidosis treated with an autologous stem cell transplant are now comparable to the US suggesting that ASCT should be more widely recommended.

5.1 Introduction

Systemic amyloidosis (AL) is a multi-system disorder characterised by tissue deposition of amyloidogenic light chains as amyloid fibrils resulting in progressive organ dysfunction and reduced survival. In the majority of cases the condition is caused by an underlying plasma cell clone, similar to Multiple Myeloma. The standard treatment of both conditions is anti-plasma cell chemotherapy. High dose myeloablative chemotherapy, as part of an autologous stem cell transplant (ASCT), remains the standard of care for younger/fitter patients with Multiple Myeloma with the potential for improved survival and a prolonged treatment free interval, and has been used for patients with AL for nearly 20 years.(108) However, ASCT is not without risk even for uncomplicated MM patients with a 2.5% overall transplant related mortality (TRM). (109) The risk of TRM is even higher for AL amyloidosis patients given the involvement of the heart, kidneys, liver and gastrointestinal tract. A TRM figure reaching 43% was reported during the early years of ASCT in AL reflecting the risk of multi-organ failure, sudden cardiac death, gastrointestinal haemorrhage and sepsis. (74) Over the last two decades a combination of stringent patient selection (110) and increased

transplant experience in the US has significantly reduced the TRM for AL amyloidosis patients to 7%,(111) this is with a 5- and 10-year survival rate of 80% and 60% respectively for those patients who achieve a haematological response (HR) to ASCT.(112) (113) Due to the complexity of patient selection, level of experience of the transplant centre and availability of high effective novel agent chemotherapy, ASCT has remained controversial in AL.

Due to previous concerns of TRM, ASCT and upfront ASCT is much less common for AL patients in the UK. Less than 5% of patients are treated with an ASCT in UK, although 10-15% would be potentially eligible. (114) This is in stark contrast to the US where approximately a third of patients are treated with an ASCT. (115) Over a ten year period, 1994-2004, a total of 92 patients were treated with an ASCT in the UK (out of a total of over 1500 patients seen);(116) this compares with 421 patients treated at a single US centre in Boston over a similar period.(117)(7) Our UK analysis 2003-2012 also suggested that an improvement in TRM lags behind that of the US (6.8% vs 5% in the UK and US respectively).(110, 118) Since this last analysis (110) multiple highly effective Bortezomib based chemotherapy combinations have been introduced with a corresponding improvement in the survival outcomes for patients with AL amyloidosis. (115) This has prompted this retrospective analysis of the TRM as well as long-term survival outcomes of all AL amyloid patients treated with an ASCT over a twenty-four-year period (1994-

2018). Our aim was to analyse survival outcomes in time cohorts to assess if there has been an improvement in TRM and survival outcomes over time.

5.2 Methods

The National Amyloidosis Centre provides a tertiary referral service for patients with amyloidosis and related disorders in the UK. The target population is all English and Scottish patients with both suspected and histologically demonstrated amyloidosis.(119) We searched our database of 5,112 patients for all patients treated with an ASCT from 1994-2018. Patients were excluded if they had been treated with an ASCT prior to their diagnosis with AL amyloidosis or if they were treated with a second ASCT. Patients were analysed as an entire cohort, and then by four time cohorts, determined by the date of ASCT: group 1: 1994-2000, group 2: 2000-2006, group 3:2007-2012 and group 4: 2013-2018; these intervals were associated with significant changes in treatment paradigms for AL amyloidosis and are similar to those reported by colleagues in the US.(120) A diagnosis of amyloidosis was confirmed by Congo red staining of a tissue biopsy with demonstration of characteristic birefringence under cross-polarized light. The amyloid subtype was confirmed by immunohistochemistry with specific antibodies, or by mass spectrometry.(121) Hereditary amyloidosis was excluded by gene sequencing as appropriate. All patients had a detailed baseline assessment of organ function with biomarker assessments and

imaging including SAP scintigraphy. Organ involvement was defined according to the international amyloidosis consensus criteria.(50) We recorded if treatment was given prior to the stem cell transplant, in addition to the ASCT conditioning regimen. Haematological response was assessed at six months. Due to the lag in organ response, organ responses were assessed at 12 months. Both were calculated from the date of the ASCT and defined according to the international amyloidosis consensus criteria.(50) For the time cohorts prior to 2012 (group 1: 1994-2000, group 2: 2000-2006, and a few patients in group 3: 2007-2012), haematological response was evaluated using the paraprotein (M protein) in cases where a serum free light chain (FLC) analysis was not available, as per the 2005 consensus criteria.(50) Cardiac response by biomarkers was not evaluable for cohorts 1 and 2 as N-terminal B natriuretic peptide (NT-proBNP) levels have only been routinely assessed in our centre since 2007. The primary outcome was TRM (defined as all-cause mortality before day +100, calculated from the return of stem cells) and overall survival (OS) following ASCT, defined as time from ASCT to death. Survival from the date of diagnosis with amyloidosis was also calculated. Secondary outcomes included: time to next treatment (defined from date of ASCT to the start of next treatment) and haematologic responses to ASCT as well the impact of depth of response on survival.

Statistical analysis was performed using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY:

IBM Corp).and Stata (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). The distribution of each numerical variable across the time cohorts was compared using the Kruskal-Wallis test. If significant, this was followed by Bonferroni corrected pairwise comparisons. The Chi-squared or Fisher's exact test, as appropriate, was used for categorical variables. Univariable logistic regression, with each variable as the outcome, was also used to determine differences between the time cohorts. The Cox proportion hazards model on the entire cohort was used to assess predictors of OS and TTNT with the assumption of proportional hazards verified. Univariable Cox regression analyses were followed by backwards stepwise Cox regression to create a multivariable Cox model. Kaplan-Meier survival curves were drawn. A significance level of 0.05 was used for all hypothesis tests. Approval for analysis and publication was obtained from the institutional review board at the University College London, and written consent was obtained from all patients in accordance with the Declaration of Helsinki.

5.3 Results

Two hundred and sixty-four patients were identified. The patient characteristics are outlined in Table 5.1.

Table 5.1: patient baseline characteristics and a comparison of variables across the time cohorts**.**

Variable	***All patients (n=264) n(%)/ median (range)***	***1994-2000 (n= 64) n(%)/ median (range)***	***2001-2006 (n=44) n(%)/ median (range)***	***2007-2012 (n=65) n(%)/ median (range)***	***2013-2018 (n=91) n(%)/ median (range)***	***P value Kruskal- Wallis /Chi-square***
Age at time of ASCT in years (range)	57 (30-70)	53(34-66)	53 (30-67)	60 (40-68)	58 (38-70)	**<0.001**
Gender male (%)	147(55.7)	37(58)	23(52.3)	37(56.9)	50(54.9)	0.943
Diagnosis to ASCT months (range)	12(0-263)	7.5(0-70)	13 (1-76)	14 (4-263	13 (1-157)	**<0.001**
Performance status						
0	101(38.3)	29(45.3)	22(50)	15(23.1)	35(38.5)	**0.002**
1	121(45.8)	17(26.6)	16(36.4)	43(66.2)	45(49.5)	
2	34 (12.9)	15 (23.4)	4 (9.1)	7 (10.8)	8 (8.8)	
3	7 (2.7)	3 (4.7)	2 (4.5)	0	2 (2.2)	
4	1 (0.4)	0	0	0	1 (1.1)	
Mayo Stage	*(n=144)*	*(n=13)*	*(n= 11)*	*(n=30)*	*(n=90)*	0.256
1	63(43.8)	6(46.2)	3(27.2)	13(43.3)	41(45.6)	
2	56 (38.9)	2(15.4)	6(54.5)	11(36.7)	37(41.1)	
3	25(17.4)	5(38.5)	2(18.2)	6(20.0)	12(13.3)	
Number of organs involved (median)	2 (1-4)	2 (1-4)	2 (1-4)	2 (1-4)	1 (1-3)	**<0.001**
Organ involvement						
Heart	85(32.2)	18(28.1)	17(38.6)	18(27.7)	32(35.2)	0.514
Kidney	180(68.2)	43(67.2)	35(79.5)	39 (60)	64(70.3)	0.182
Liver	52(19.7)	6(15.4)	7(19.4)	2 (3.1)	8 (8.8)	**0.030**
GI	10(3.8)	0	4 (9.1)	4 (6.2)	2 (2.2)	0.056
Peripheral NS	21(8.0)	1(1.6)	6 (13.6)	7 (10.8)	7 (7.7)	0.101
Autonomic NS	10(3.8)	0	1 (2.3)	4 (6.2)	5 (5.5)	0.210
SFLC(mg/L) (range)	*(n=235)*	*(n=38)*	*(n=35)*	*(n=65)*	*(n=91)*	
Lambda	N/A	57 (0-4320)	49(1-5180)	104(0-26702)	86(2-12800)	0.515
Kappa	N/A	10(1-5310)	16(0-6810)	14(1-3040)	10(0-2211)	0.220
IVS (mm)	11(7-20)	11 (7-20)	12 (8-16)	11 (8-16)	11 (8-19)	0.257
NT-proBNP (ng/L)	*(n=243)*	*(n=51)*	*(n=38)*	*(n=64)*	*(n=90)*	0.455
	318(1-92773)	574(8-92773)	364(1-23756)	262(25-5636)	385(17-22025)	
Baseline creatinine(µmol/l)	78(34-654)	85(44-500)	84 (53-480)	75 (34-654)	72 (42-490)	**<0.001**
Albumin (g/l)	33(12-52)	31(12-52)	35 (17-49)	39 (17-51)	32 (16-49)	**0.004**
Proteinuria (g/dl)	3.8(0-20)	4.2 (0-20)	4.2(0.1-15)	1.15 (0-12)	5 (0-20)	**0.012**
Bilirubin(µmol/l)	7(1-48)	8 (2-48)	7 (4-12)	7 (1-16)	6 (2-27)	**0.004**
ALP (IU/l)	80(36-986)	90 (36-986)	92 (41-491)	71 (43-306)	76(37-795)	**0.002**

ASCT line of treatment	2 (1-5)	1 (1-2)	2 (1-4)	2 (1-4)	2 (1-5)	**<0.001**
Pre-ASCT regimen						**<0.001**
Thalidomide	58(36.3)	Missing/ not given	4(14.8)	39(60)	15(16.5)	**<0.001**
Velcade	83(51.8)		16(59.3)	12(18.8)	55(60.4)	
Melphalan	6 (3.8)		2 (7.4)	7 (15.6)	22(24.2)	
Other	13 (4.9)		5(18.5)	6 (9.4)	3(3.3)	
Missing/ not given	104(39.4)		17(38.6)	0	0	
TRM (n (%))	23(8.7)	12(18.8)	6 (13.6)	4 (6.2)	1 (1.1)	**0.001**
Median OS post ASCT (months)	87	60	60	Not reached	Not reached	
TTNT (months)	24(0-187)	87 (2-187)	18 (3-132)	28 (0-68)	15 (0-45)	

ASCT= autologous stem cell transplant; GI= gastrointestinal; IVS= left ventricular septum; ALP= alkaline phosphatase; NT-proBNP= N- terminal B natriuretic peptide; SFLC= serum free light chain; TRM= transplant related mortality; OS=overall survival; TTNT= time to next treatment

Differences in patient and disease characteristics across the time cohorts

The variables that significantly differed across the time cohorts were: age at time of ASCT, time from diagnosis with amyloidosis to date of ASCT, number of organs involved with amyloidosis, baseline creatinine, albumin, proteinuria, bilirubin, alkaline phosphatase (ALP) and the number of lines of chemotherapy treatment prior to ASCT. Pairwise comparisons, with each variable taken as the outcome, were then used to identify the time cohorts between which the

variables differed significantly (see Appendix, Table 6). Patients in the latter two-time cohorts (median age 60 years and 58 years for cohorts 3 and 4 respectively) were significantly older at time of transplant than the earlier cohorts (median age 53 years for cohorts 1 and 2). The performance status of transplanted patients also significantly differed over time, the majority of patients in the early cohorts 28% vs 12% of patients ECOG 2 or above in cohorts 1 vs 4 respectively, P=0.030. A major difference was that in the latter two cohorts, compared with the first two-time cohorts, a higher proportion of patients had received some chemotherapy prior to proceeding to transplant (81% and 88% vs. 57% and 0% respectively P= <0.001). This was also reflected in a difference in the time from diagnosis with amyloidosis to ASCT which was a median of 7.5 months in cohort 1 vs. 13 months (P=0.001) for cohort 3, and 14 months for cohort 4 (P= 0.002).

A full list of the variables that differed across time cohorts, the cohorts between which the variable differed and the corresponding P values for this difference are provided in appendix table 1.

Pre-ASCT treatment and conditioning

Eighty patients (n=80, 30%) had an up-front ASCT, 59% (n=47/80) of which were in the first cohort (1994-2000). The majority of patients had their ASCT after first line treatment (n=118, 45%). The

most frequently used regimen pre-ASCT was a Bortezomib based regimen (n=83, 51.8%), followed by Thalidomide (n=58, 36.3%).

A reduced melphalan conditioning dose was used in 43% of patients (n=80/186) (melphalan dose details were not available in 72 cases) and six patients (3%) received an alternative conditioning regimen with carmustine, etoposide, cytarabine and melphalan (BEAM). A significant difference in the conditioning regimen was only seen between the earliest two cohorts, with a dose reduction used in 28.6% vs 62.5% in cohort 1 vs 2 respectively, P=0.004.

Treatment related mortality (TRM)

With a median follow-up of 68 months (range 2-284 months), there were 106 deaths (40.2%). TRM was defined by death within 100 days of return of stem cells. The TRM for the entire cohort was 8.7% (N=23/264). The TRM significantly reduced over time, 18.8% vs 13.6% vs 6.2% vs 1.1% for cohorts 1, 2, 3 and 4 respectively, (P = 0.004).

Response assessment

Haematological responses are outlined in Table 5.2.

Table 5.2: Number (%) with haematological response to autologous stem cell transplant, determined at six months.

Response (n=evaluable)	**All patients (n=264)**	**1994-2000 (n=64)**	**2001-2006 (n=44)**	**2007-2012 (n=65)**	**2013-2018 (n=91)**
Haematological response	*(n=236)*	*(n=46)**	*(n=35)*	*(n=65)*	*(n=90)*
Complete response	122 (52.4)	32 (69.6)	13 (37.1)	31(47.7)	46 (51.1)
Very good partial response	40 (17.2)	3 (6.5)	4 (11.4)	11(16.9)	22 (24.4)
Partial response	59 (25.3)	9 (19.6)	16 (45.7)	23 (35.4)	11 (12.2)
No response	10 (4.3)	2 (4.3)	2 (5.7)	0 (0)	6 (6.7)
Progressive disease	5 (2.1)	0 (0)	0 (0)	0 (0)	5 (5.6)
Cardiac response	*(n=46/85)*	**	**	*(n=16/18)*	*(n=2732)*
Response	28 (60.9)			8 (50)	*18 (66.7)*
No response	10 (21.7)			5 (31.3)	*4 (14.8)*
Progression	8 (17.4)			3 (18.8)	*5 (18.5)*
Renal response	*(n=134/180)*	*(n=22/43)*	*(n=26/35)*	*(n=33/39)*	*(n=53/64)*
Response	101 (76.0)	9 (40.9)	11 (42.3)	17 (51.5)	38 (71.7)
No response	18 (13.5)	10 (45.5)	11 (42.3)	10 (30.3)	14 (26.4)
Progression	14 (10.5)	3 (13.6)	4 (15.4)	6 (18.2)	1 (1.9)
Liver response	*(n=32/52)*	*(n=10/23)*	*(n=8/15)*	*(n=5/5)*	*(n=9/10)*
Response	7 (13.5)	2 (20)	1 (12.5)	0 (0)	4 (44.4)
No response	19 (61.3)	6 (60)	5 (62.5)	3 (60)	5 (55.6)
Progression	6 (19.4)	2 (20)	2 (25)	2 (40)	0 (0)

* Assessed by SFLC in 59% (n=27/46) and paraprotein in 41% (n=19/46)

** Cardiac response assessment not possible due to lack of NT-proBNP levels prior to 2007.

Of the entire cohort of 264 patients (88%, N=236/264) were evaluable (26 patients had died and five had missing reading). The responses were as follows: a complete response (CR) (n=122, 52%), a very good partial response (VGPR) (n=40, 17%), a partial response (PR) (n= 59, 25%), no response (NR) (n=10, 4%) and progressive disease (PD) (n=5, 2%). An overall haematological response (defined as a partial response or better) was seen in 95% of all cases

(n=221/233) and by subset analyses: cohort 1: 97% (n=44/46), cohort 2: 94% (n=33/35), cohort 3: 100% (n=65/65), cohort 4: 88% (n=79/90). An overall haematological response for cohorts 3 and 4 combined was achieved in 93% of patients with CR: 50% (n=77/155), VGPR: 21% (33/155), PR: 22% (34/155). The rates of VGPR/CR were greatest for cohorts 1 (76%, n=35/46) and cohort 4 (76%, n=68/90) compared with cohorts 2 (49%, n=17/35) and cohort 3 (65%, n=42/65), but this will be biased (with over representation of CR) as a response assessment using serum free light chain was only possible in 59% (n=27/46) of patients in this early cohort with haematological response based on M-protein reduction in the remaining 41% (n=19/46). Organ responses at 12 months are outlined in table 5.3.

Table 5.3: Univariable Cox regression analysis of overall survival for the entire cohort (n=264)

Variable	**Hazard Ratio**	**95% CI**	**P value**
Age at time of ASCT (years)	0.991	0.965-1.018	0.520
Gender (male)	1.143	0.740-1.764	0.548
Diagnosis to ASCT (months)	1.007	0.998-1.016	0.146
Performance Status (compared to 0) (n=264) 1 2, 3 or 4	 0.640 2.647	 0.389-1.051 1.466-4.781	 0.078 **0.001**
Mayo Stage (compared to 1) (n=144) 2 or 3	 2.620	 1.038-6.613	 **0.042**
LC type (n=235) kappa/lambda	 1.263	 0.737-2.163	 0.396
Number of organs involved (median)	1.592	1.270-1.994	**<0.001**
Amyloid load on SAP scintigraphy (n=238) Equivocal Small Moderate large	 1.335 0.832 1.428 1.950	 0.560-3.180 0.430-1.610 0.701-2.911 0.960-3.961	 0.514 0.586 0.327 0.065
Organ Involvement (n=264) Heart Kidney	 1.565 1.535	 1.008-2.429 0.935-2.521	 **0.046** 0.090

Liver GI Peripheral nervous system Autonomic nervous system	2.287 2.810 0.475 0.631	1.261-4.147 1.207-6.543 0.190-1.188 0.369-1.080	**0.006** **0.017** 0.112 0.093
IVS (mm)	1.204	1.083-1.338	**0.001**
Log-NT-proBNP	1.811	1.269-2.584	**0.001**
Log-creatinine (µmol/l)	3.168	1.201-8.359	**0.020**
Albumin (g/l)	0.980	0.956-1.005	0.113
Proteinuria (g/dl)	1.026	0.976-1.078	0.314
Bilirubin (µmol/l)	1.004	0.940-1.072	0.912
Abnormal ALP (> 129IU/l)	1.927	1.174-3.165	**<0.010**
ASCT line of treatment (compared to 1) (n=264) 2/3/4	0.600	0.381-0.947	**0.028**
Pre-ASCT regimen Thalidomide (n=58) Velcade (n=83)	 0.581 1.236	 0.303-1.115 0.655-2.333	 0.102 0.513
Conditioning (compared to Melphalan 200) Dose reduced melphalan (n=80)	1.263	0.803-1.987	0.313

ASCT= autologous stem cell transplant; LC= light chain; IVS= left ventricular septum; NT-proBNP= N-terminal pro-B natriuretic peptide; ALP= alkaline phosphatase.

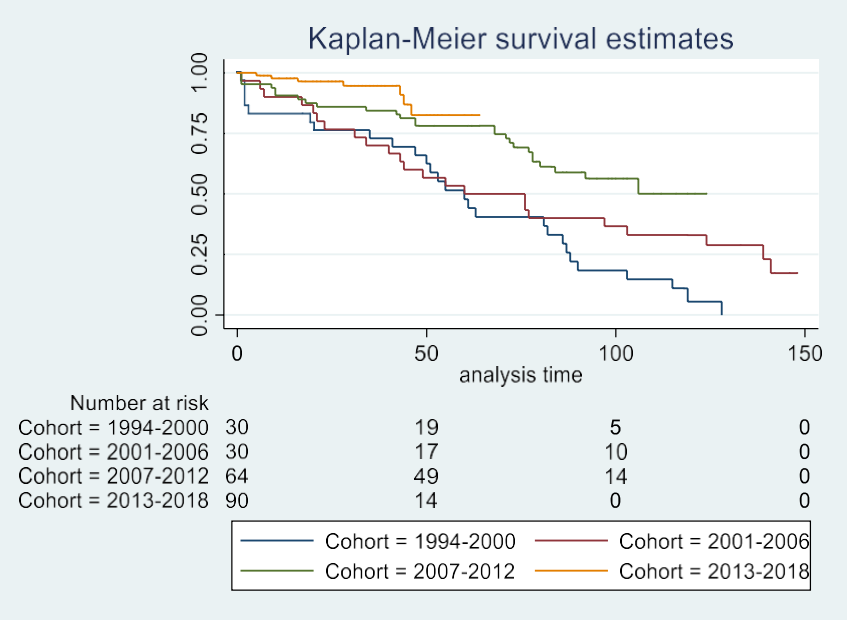
Of the 85 patients with cardiac involvement, a cardiac response was evaluable at 12 months in 54% (n=46/85), the remaining patients had died or had missing NT-proBNP values. A cardiac response was seen in 61% (n=28/46). Of the 180 patients with renal involvement 74% (n=133/180) were evaluable, the remaining 47 (26.1%) of patients had died or had missing data. A renal response was seen in 76% (n=101/133) of cases. Fifty-two patients had liver involvement and 60% (n=31/52) had data for an organ assessment at 12 months. The majority of patients had neither a liver response nor progression (61%, n=19/31).

Overall Survival (OS)

All survival analyses are with a cut-off of 150 months, given the low number of patients at risk beyond this time point. The median overall

survival from diagnosis with AL amyloidosis was 99 months (95% CI: 87-118 months) and by time cohort: cohort 1: 49 months (95% CI: 15-69 months); cohort 2: 51 months (23-106 months); cohort 3: 137 months and cohort 4, not reached. The median OS from ASCT, was 87 months (95% CI: 77-106 months) for all patients and by time cohort: 60 months (95% CI: 41-86 months) for cohorts 1 and 2 (95% CI: 40-103 months) and not reached in the latter two cohorts (Fig. 5.1).

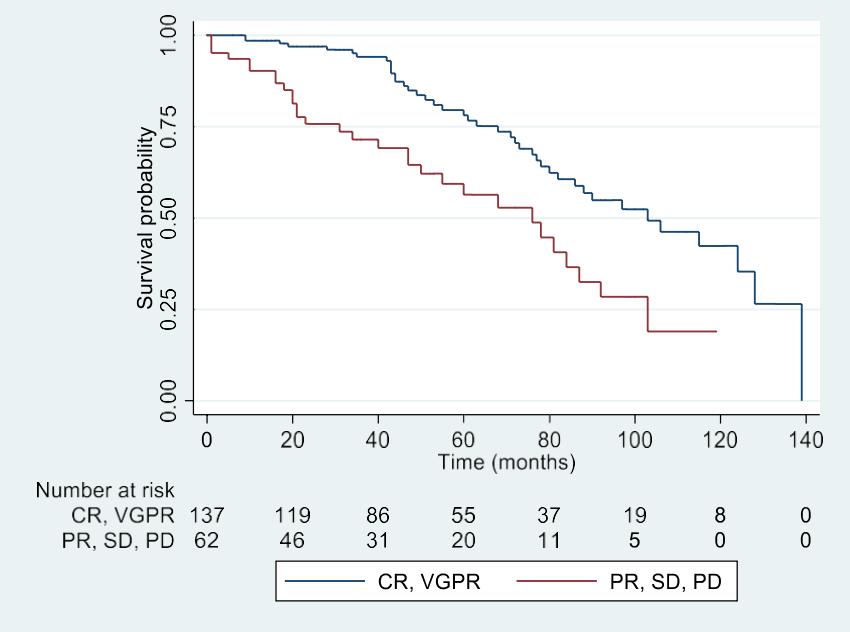
Figure 5.1 Overall survival by date of autologous stem cell transplant: blue line= 1994-2000, green line =2001-2006, red = 2007- 2012, yellow= 2013-2018.



The OS at 5, 8 and 10 years from diagnosis for the entire cohort was 74% (95% CI: 67-79%), 56% (95% CI: 48-64%) and 44% (95% CI: 35-53%), respectively. The 5-year survival for cohorts 3 and 4

were: 83% (95% CI 72-90%) and 90% (95% CI: 78-96%), respectively. Haematological response was a strong predictor of outcome (Fig. 6.2), with a median OS of 139 months (95% CI: 82-139 months) for patients who achieved a CR or VGPR compared with 64 months (95% CI: 50-92 months) for those in a PR, SD or PD at six months, (P= 0.007).

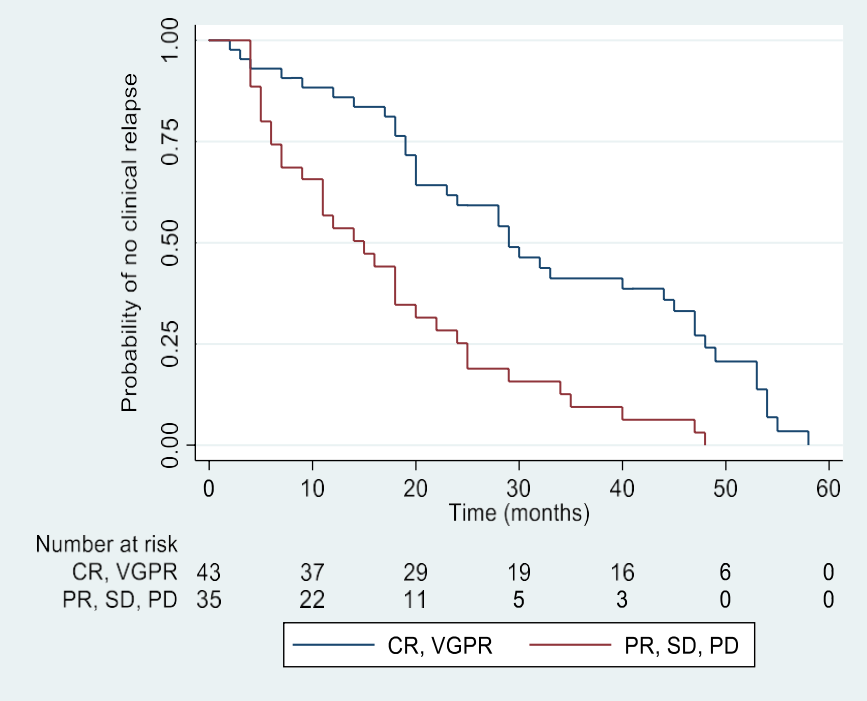
Figure 5.2 Overall survival by haematological response at 6 months: blue line = complete response and very good partial response, red= partial response, no response and progressive disease, P= <0.007.



We then assessed the outcome of patients achieving a CR versus VGPR for the last two cohorts. The median OS was not reached in either cohort and there was no significant difference in the OS

between those who achieved a CR vs VGPR for cohort 3 (P= 0.255) or cohort 4 (P=0.665). Haematological response also predicted time from ASCT to next treatment (TTNT). For the entire cohort the TTNT was a median of 48 months (95% CI: 18-29 months) for the entire cohort. There was a significant impact of TTNT when analysed by depth of haematological response: CR/ VGPR: 49 months (95% CI: 24-48 months) compared with PR/SD/PD: 35 months (95% CI: 9-20 months), P<0.0001, (Fig. 5.3).

Figure 5.3 Time to next treatment by haematological response: blue= complete response and very good partial response, red= partial response and no response, P=<0.0001.



Further stratification showed median TTNT was: CR: 54 months (95% CI: 47-73 months), VGPR: 73 months (95% CI: 19- months), PR: 24 months (95% CI: 12-35 months) and SD: not evaluable. The

TTNT was apparently lower for CR than VGPR, but this may represent bias due to a lack of FLC measurements in the early cohorts. To overcome this, we analysed cohort 3 and 4, where data on FLC response was more complete; the median TTNT was 53 months versus 19 months for those patients who achieved a CR versus VGPR in cohort 3, but this was not significant, (P=0.204) and the median TTNT was not reached for either group in cohort 4.

Table 5.3 outlines the results of the univariable Cox regression analysis, looking for variables that were prognostic of OS post ASCT. The factors that significantly predicted survival at the 5% level (P<0.05) on univariable analysis were: performance status of 1 or above, Mayo stage 2 or 3, the number of organs involved with amyloidosis, cardiac involvement, gastrointestinal involvement (GI), liver involvement, left ventricular wall thickness (IVS), log-N-terminal B natriuretic peptide (NT-proBNP), log-creatinine, elevated ALP (defined as a value >129IU/L) and having two lines of treatment prior to ASCT. A multivariable model (Table 6.4) was produced by backward stepwise Cox regression analysis on all variables except cardiac involvement and IVS, given the overlap of these two variables with Mayo stage. Independent predictors of OS on multivariable analysis were Mayo stage 2 or 3 (P= 0.004), an abnormal ALP (P<0.001) and liver involvement as defined by the standard consensus criteria (P= 0.014). (50)

Table 5.4: Multivariable Cox regression analysis of overall survival for the entire cohort

	Hazard ratio	95% CI	P-value
Performance status	0.43	0.17- 1.08	0.071
Abnormal ALP	4.36	1.68-11.32	**0.002**
Mayo stage 2 or 3	6.76	1.84-24.71	**0.004**
ASCT line treatment	0.47	0.17- 1.28	0.139
GI involvement	4.60	0.55- 38.44	0.159
Log creatinine	3.91	0.77-19.78	0.099
Liver involvement	6.42	1.44-28.53	**0.014**

NT-proBNP = N-terminal B natriuretic peptide; ALP= alkaline phosphatase; TTNT= time to next treatment; CI= confidence interval

5.4 Discussion

This study describes the outcome of 264 UK AL amyloidosis patients treated with an ASCT over a 24-year period, 1994-2018. An improvement in the safety of ASCT is seen, with a significant reduction in TRM over time. The most recent cohort of patients had a haematological response rate of 88% and a 5-year OS of 90% suggesting that ASCT is not only an increasingly safe but highly efficacious treatment for AL amyloidosis patients.

ASCT has been utilised as a treatment for patients with AL amyloidosis for over two decades.(108) The high initial TRM of between 10-43% (74) resulted in efforts to better understand patient, disease and transplant related factors which may be linked to an increased risk of mortality. This has, to a large extent, been led by detailed analyses of the experience from large US transplant centres to identify factors that are predictive of TRM and has shaped global

practice. Unsurprisingly, cardiac involvement was identified as the main predictor of mortality in AL patients undergoing ASCT.(122) The number of visceral organs involved with amyloidosis and the serum creatinine at the time of transplantation were also found to be predictive of poor outcome. (123) In 2002, the Mayo group combined these prognostic variables to categorise patients into good, intermediate and poor risk groups based on the number of organs involved, the presence of cardiac involvement and renal impairment.(124) The Mayo staging system, outlined in 2004 and revised in 2012, prompted the refinement of this selection criteria to incorporate the cardiac biomarkers with the presenting difference in serum free light chain (dFLC);(63) a cardiac troponin (cTNT) > 0·06 ng/ml or NT-proBNP > 5000ngl were considered to be markers of high risk of early TRM.(118) The analysis from our group identified: severe autonomic neuropathy, significant GI bleeding due to amyloid, advanced renal failure, age over 70 years, symptomatic recurrent amyloid related pleural effusions, and a poor performance status (ECOG >2) (125) and nephrotic range proteinuria (110) should be considered as contraindications to ASCT.

We were also able to study prognostic factors of OS in this study. Creatinine and the number of organs involved were significant on univariable but not subsequent multivariable analysis. Cardiac involvement, particularly the severity as defined by the 2004 Mayo stage, was the most important predictor of survival on univariable and multivariable analyses (P=0.041) in this study. All the other

cardiac variables (NT-proBNP, cardiac involvement and IVS) were significant on univariable analyses, but were not included in subsequent multivariable analyses given the overlap with Mayo stage. The prognostic impact of Mayo staging is unsurprising and reinforces the critical utility of the Mayo staging system in stratifying patients, supporting its continued use to select patients for an ASCT.(126) An abnormal ALP and liver involvement, using the standard definition of liver involvement of ALP x 1.5 ULN, were also prognostic on multivariable analyses. We have previously identified liver involvement to be an independent predictor of mortality in AL.(120, 127). The confirmation of this finding in the current cohort is of particular importance since detailed assessment of liver involvement has no place in current ASCT selection criteria, but most patients with substantial liver amyloid deposits have multiorgan amyloid deposition and liver involvement is likely to simply be a barometer for the total body amyloid burden.

These efforts from international centres, as well as our previous studies to carefully define factors that indicate increased risk of morbidity and mortality during ASCT, have resulted in progressively stringent patient selection. This study confirms that, even when transplants are undertaken across multiple transplant centres, this has significantly helped to reduce the TRM over time, from 18.8% in 1994-2000 to 1.1% from 2013-2018. This latest TRM compares favorably to a 2.4% TRM quoted by the US for the period 2010-2016 (120) and also to the 2.1% quoted in a recent nationwide study

from the Danish Multiple Myeloma registry for multiple myeloma treated with ASCT.(4)

The subset analyses of this study highlight how patient characteristics have changed over time. The median age at time of transplant has significantly increased over time. Rather than advancing age being protective against TRM, this may suggest that ASCT is increasingly being offered to older patients, perhaps reflecting increasing experience and confidence in ASCT. There was a significant trend away from transplanting patients in a poor performance category (28% vs 12% of patients ECOG 2 or above in cohorts 1 vs 4 respectively, P=0.030). Performance status is a known risk factor for TRM (118) and this reinforces that performance status rather than age is a more reliable factor when determining whether an ASCT is appropriate. Whilst the Mayo stage (or cardiac involvement) remained the strongest predictor of OS, the proportion of patients with cardiac involvement did not significantly differ over time in this study. Likewise, the proportion of patients in various Mayo stages did not significantly differ across the cohorts. However, the key difference was a very high proportion of patients in the latter cohorts had some prior chemotherapy. This may well work as a "stress test" for selecting patients as most of the early mortality in AL occurs in the first few months following diagnosis.(115)

In this current era of novel agent-based therapy, the major advantage

of ASCT is better long-term outcomes. Our previous studies, possible tempered by higher TRM, seemed to suggest less long-term benefits of ASCT in the UK patient population compared to outcomes reported from large US series. The Boston experience described the outcome of 629 patients with AL amyloidosis treated with ASCT over the period 1994-2014 with a median OS of 7.63 years,(120) which exceeded the UK median survival of 5.3 years reported in 1994-2004,(116) and 4 years in 2003-2012.(110) This updated analysis of UK outcomes is very encouraging and suggests that ASCT UK outcomes are now comparable (Table 5.5). The median OS for the entire UK cohort of 9.6 years is comparable to that reported by the Boston group over a similar 20-year period.(112) Our finding also supports that of the Boston and Mayo groups, that haematologic

Table 5.5: Comparison with contemporary data. All values are percentages, unless otherwise specified.

	Cohort 1	**Cohort 2**	**Cohort 3**	**Cohort 4**
TRM				
D'Souza et al. (2015)	20	11	5	
Sidiqi et.al. (2018)	14.5	8.6	2.4	
Sanchorawala (2015)	14.0	8.4	7.5	
Current UK cohort	18.8	13.6	6.2	1.1
5 yr. OS				
D'Souza et al. (2015)	55	61	77	NA
Sidiqi et.al. (2018) (median)	75 months	120 months	Not reached	
Sanchorawala (2015) (median)	57 months	90 months+	92 months	
Current UK cohort	45	51	83	90
Haematologic response				
D'Souza et al. (2015)	NA	68	80	84
Sidiqi et.al. (2018)	69	79	84	
Sanchorawala (2015)	NA	NA	NA	
Current UK cohort	96	94	100	88

Haematology response= partial response or better; TRM= transplant related mortality; OS= overall survival; UK= United Kingdom

*D'Souza et.al (2015) cohorts 1: 1995-2000; cohort 2: 2001-2006; cohort 3: 2007-2012

** Sidiqi et. al (2018) cohorts: 1: 1996-2002, cohort 2: 2003-2009; cohort 3: 2010-2016

*** Sanchorwala (2015) cohorts: 1: 1994-2000, cohort 2: 2000-2009, cohort 3: 1994-2014

**** Current UK cohort: 1: 1994-2000; cohort 2: 2001-2006; cohorts 3: 2007-2012; cohort 4: 2013-2018.

response to ASCT is an important predictor of outcomes as the median OS and TTNT in this study were significantly longer for patients who achieved a CR or VGPR compared with those who achieved a PR or were non-responders.

This study is not without its limitations. This is a retrospective study with data collected over a twenty-four-year period. The National Amyloidosis Centre provides a tertiary referral service for patients with amyloidosis and related disorders in the UK. The target population is all English and Scottish patients with suspected and histologically demonstrated amyloidosis,(119) however patients are highly selected by the requirement to attend. The referring local hospitals were the treating centres for the ASCT and so we were unable to reliably collect ASCT associated toxicity data and the melphalan conditioning dose was missing in several cases. There was also missing data (SLFC and NT-proBNP) particularly from the earlier cohorts. Despite these limitations, this study of 264 AL amyloidosis patients treated with an ASCT represents the largest UK analysis of the long-term survival outcomes to date.

There are still unanswered questions regarding ASCT for AL patients. Whether induction therapy is required for AL amyloidosis patients remains controversial. The majority of AL patients will have 10% or fewer clonal plasma cells within the marrow and so there is no need for 'induction' therapy or chemotherapy to 'debulk,' or reduce the plasma cell burden .(124) Upfront ASCT approach is frequently used in the US where 90% of patients have an ASCT within 12 months of diagnosis and 69% within 6 months.(113) The median time from diagnosis to ASCT in this study was 12 months and ASCT was most commonly following chemotherapy. There was also a notable trend in this study away from up-front ASCT, with an increasing median time from diagnosis to ASCT. Superior haematological response rates, PFS and OS have been reported with immunomodulatory/ proteasome inhibitor therapy prior to ASCT, (128) as the majority of our patients received in the later cohorts. This trend towards giving chemotherapy prior to ASCT may help towards explaining the decreased TRM. This may be a critical factor in improvement in outcomes and raises a critical question: should the field move away from upfront transplants for AL in favour of pre-treating prior to transplant? This is further reinforced by prospective data from colleagues in Boston showing remarkable improvement in haematologic response rates for patient treated with Bortezomib-dexamethasone prior to ASCT.(129) Lately, we reported the efficacy and safety of 'truly' deferred ASCT. (114). This method permits

substantial organ recovery prior to transplant and may allow more patients to be eligible for this effective treatment option. However, the Boston series of induction chemotherapy resulted in 15% of patients being unable to proceed to transplant due to a change in their clinical status. There is not enough data to formally study the approach of deferring an ASCT and treating patients with 'induction chemotherapy' prior to ASCT requires further exploration.(130)

In conclusion, this study of 264 patients with AL amyloidosis treated with an ASCT over a 24-year period confirms the improved safety of this treatment over time, with figures comparable to contemporary international data. This study supports the continued use of ASCT for patients with AL amyloidosis. The current analysis confirms the validity of section criteria and suggests that Mayo stage, liver involvement and performance status are particularly important criteria for selecting patients eligible for this procedure. Patients who achieve a deep haematological response to ASCT appear to benefit the most with a prolonged clinical remission and excellent long-term survival outcomes.

Chapter Six

Autologous stem cell transplantation versus bortezomib alone for light chain amyloidosis

This chapter is written in the context of my publication: Autologous stem cell transplantation vs bortezomib based chemotherapy for the first-line treatment of systemic light chain amyloidosis in the UK. Sharpley FA, Manwani R, Petrie A, Mahmood S, Sachchithanantham S, Lachmann HJ, Martinez De Azcona Naharro A, Gillmore JD, Whelan CJ, Fontana M, Cohen O, Hawkins PN, Wechalekar AD. Eur J Haematol. 2021 Apr;106(4):537-545. doi: 10.1111/ejh.13582. Epub 2021 Jan 27. PMID: 33460466.

Key Points:

- ASCT confers no survival advantage over bortezomib therapy with comparable overall survival.
- Deep haematological and organ responses can be achieved with transplantation, but patients who do not achieve an adequate clonal response to ASCT may benefit more from standard bortezomib therapy.

6.1 Introduction:

High dose chemotherapy followed by an autologous stem cell

transplantation (ASCT) is a highly effective treatment for patients with AL amyloidosis. The median overall survival (OS) for patients who achieve a haematological complete response to treatment is in excess of 15 years.(113) The alternative to ASCT is standard chemotherapy. The median survival with oral melphalan and dexamethasone is 5.1 years,(131) and although an initial European collaborative study of bortezomib, cyclophosphamide and dexamethasone demonstrated encouraging findings, only 55% of patients were predicted to be alive at 5 years.(132) And so ASCT is considered to be the treatment of choice for those patients eligible for this procedure. Despite this, there has been a long-standing debate of the benefit of ASCT over standard chemotherapy. The major studies trying to address this question to date are outlined in Table 6.1.

Table 6.1: an outline of the major studies to date addressing the question of ASCT versus conventional chemotherapy for the treatment of patients with AL amyloidosis

Study author and year	**Patient numbers (n)**	**OS ASCT**	**OS conventional chemotherapy**	**PFS ASCT**	**PFS vs conventional chemotherapy**
Dispenzieri et.al (2004)(133)	126	71%	41%	/	
Gertz (2016)(134)	89	83.6%	58.5%	51.7%	29.1%
Oke et. al (2017)(111)	74	74 months	8 months	Not reached	9 months
Jaccard, et. al (2007)(135)	100	22.2 months	56.9 months	32.5 months	32 months
Sharpley et. al (2019)	136	103 months	Not reached	50 months	42 months

OS= overall survival; PFS= progression free survival; ASCT= autologous stem cell transplant

Dispenzieri et.al (2004) performed a case-control study of 63 patients with AL amyloidosis which demonstrated a superior four-year OS for ASCT compared to standard chemotherapy (71% vs. 41% respectively).(133) In 2007 the French group also performed a prospective, randomised trial comparing ASCT to oral mephalan and dexamethasone therapy but found inferior survival outcomes for ASCT (median OS 22.2 vs. 56.9 months, respectively). This controversial finding was attributed to the high transplant related mortality (TRM) and the inclusion of patients with severe cardiac amyloidosis,(135) but in 2009 a systematic review of 12 studies supported this finding.(136)

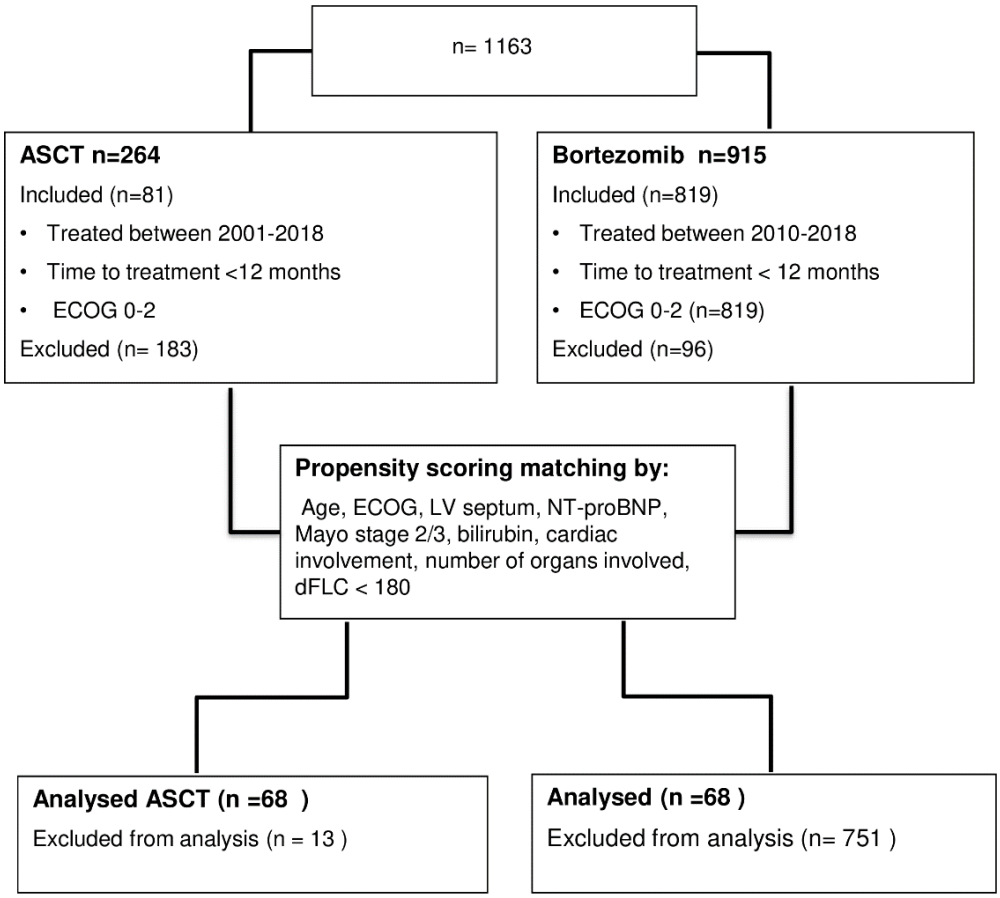
Since these initial studies, the TRM associated with ASCT has dramatically decreased due to a combination of more stringent patient selection and increased clinician experience.(113) This reduction in TRM should favour ASCT over standard chemotherapy.(118) This certainly seemed to be the case in the 2016 Mayo group analysis of 89 patients with AL amyloidosis allocated to ASCT or melphalan and dexamethasone therapy. Although the haematological response rates were comparable between the two treatment arms, the three-year progression free survival (PFS) (51.7% vs. 29.1%) and OS (83.6% vs. 58.8%) were superior for ASCT over melphalan and dexamethasone therapy, respectively. A retrospective study published in 2017 of ASCT (n=43) versus conventional chemotherapy (n=31) also confirmed

superior PFS (not reached vs. 9 months) and OS (74 months vs. 8 months) for ASCT.(111) This is the only study to date comparing the outcomes of ASCT with patients treated with bortezomib-based therapy. Data from our own centre (unpublished, Manwani et.al) suggests that this later study vastly underestimates the survival seen with bortezomib treatment in the modern era, suggesting that outcomes may now be comparable to patients treated with ASCT. To resolve this ongoing controversial debate we performed a retrospective, case-matched study of patients with AL amyloidosis treated with an ASCT versus standard bortezomib chemotherapy in the UK.

6.2 Methods

We searched our database of 5,112 patients for all newly diagnosed patients with AL amyloidosis treated with high dose chemotherapy followed by ASCT or bortezomib treatment from 1994-2018. The consort diagram in Figure 1 illustrates the selection process.

Figure 6.1 Consort diagram to outline the selection of patients in both the autologous stem cell transplant and bortezomib treatment groups



ASCT=autologous stem cell transplant; ECOG= Eastern Cooperative Oncology Group performance status; NT-proBNP= N-terminal B natriuretic peptide; LV= left ventricle; dFLC= difference between involved and uninvolved light chains.

Patients were excluded if the date of ASCT preceded 2001, to allow a fair comparison of modern transplant practice with bortezomib treatment. All patients in the bortezomib arm received treatment from 2007-2018. Patients from both treatment arms were excluded if the time from diagnosis to treatment exceeded 12 months, or if the patients were Eastern Cooperative Oncology Group performance

status (ECOG) >2. Duplicate patients, or those patients treated with both bortezomib and ASCT were also excluded from the analysis. Patients were then matched on a 1:1 basis using a propensity scoring approach for all variables thought to clinically impact survival and be significant at the 5% level on univariable analysis, this included: age at the time of treatment, Eastern Cooperative Oncology Group performance status (ECOG) 1 or 2, N-terminal B natriuretic peptide (NT-proBNP), bilirubin, cardiac involvement, left ventricular septal wall thickness on echocardiogram (lVS), the number of organs involved, the difference in serum free light chains >180mg/L (dFLC) and Mayo stage 2 and 3, as per the 2004 criteria.(38) In all cases a diagnosis of amyloidosis was confirmed by Congo red staining of a tissue biopsy with demonstration of characteristic birefringence under cross-polarized light. The amyloid subtype was confirmed by immunohistochemistry with specific antibodies, or by mass spectrometry.(121) Hereditary amyloidosis was excluded by gene sequencing if the amyloid subtype remained unclear. All patients had a detailed baseline assessment of organ function with biomarker assessments and imaging including SAP scintigraphy. Organ involvement was defined according to the international amyloidosis consensus criteria. Haematological response was assessed at six months and organ responses at 12 months, both calculated from the start of bortezomib treatment, or from the date of return of stem cells for those in the ASCT group, and defined according to the international amyloidosis consensus criteria.(86) The primary outcome was overall

survival (OS) defined as time from bortezomib/ASCT to death in months. Survival analyses were also calculated at 12- and 48-months post treatment. To overcome the possible impact of early mortality on outcome, landmark analyses were also performed at 12- and 48-months post treatment. Secondary outcomes included: time to next treatment (TTNT), defined from the date of bortezomib/ASCT to the start of next treatment, and TRM, defined as all-cause mortality before day +100, calculated from the start of bortezomib treatment or from the return of stem cells.

Statistical analysis was performed using SPSS version 21 and Stata version 15. Survival outcomes were analysed using the Kaplan-Meier method with comparisons done using the log rank test. The Cox proportion hazards model was used to assess predictors of OS. All p-values were two sided and any variable with a P value <0.05 on univariate analysis. Approval for analysis and publication was obtained from the institutional review board at the University College London, and written consent was obtained from all patients in accordance with the Declaration of Helsinki.

6.3 Results

i) Patient baseline characteristics

A total of 68 patients were eligible for analysis in both treatment arms after propensity score matching. The baseline patient characteristics are outlined in Table 6.2. There was no significant

difference between the two groups using a propensity scoring matching approach for all variables considered to both clinically affect survival and also those variables significant on univariable analysis. This included: performance status (ECOG), Mayo stage (2004),(38) cardiac involvement, number of organs involved, difference in serum free light chains >180mg/l, age at treatment, left ventricular septal thickness, N-terminal pro-B-natriuretic peptide (NT-proBNP), high sensitivity cardiac troponin (hsTNT), bilirubin or alkaline phosphatase (ALP) (Appendix Table 2). The median time from diagnosis with AL amyloidosis to treatment was <12 months for all patients (ASCT- seven months, bortezomib-two months). All patients were treated from 2001-2018; the ASCT patients 2001-2018 and the bortezomib group 2007-2018. The stem cell conditioning regimen was available for 37% (n=25/68) patients. Full dose melphalan (300mg/m2) was given in 8 patients (32%) and reduced dose intensity melphalan (140mg/m2) in 17 patients.

Table 6.2: a comparison of patient baseline characteristics between patients treated with ASCT and bortezomib

Variable **N/median, (range/%)**	**Bortezomib (n=68)** n(%)/median(CI)	**ASCT (n=68)** n(%)/median(range) or mean(range)
Age at treatment (years)	59.9 (40-75)	58.5 (57-61)
Gender (male)	42 (56)	33 (44.0)
Performance Status		
0	29 (52.7)	26 (47.3)
1	34 (48.6)	36 (51.4)
2	5 (45.45)	6 (54.6)
Mayo Stage		
1	37(49.3)	38(50.7)
2	26(53.1)	23(46.9)
3	5(41.7)	7(58.3)
No. of organs involved	1 (1-2)	1 (1-1)
Organ Involvement		
Heart	18(48.7)	19 (51.4)
Kidney	51 (75)	46 (67.6)
Liver	10 (32.3)	21 (67.7)
GI	0 (0)	2 (100)
Peripheral NS	7 (63.6)	4 (36.4)
Autonomic NS	6 (85.7)	1 (14.3)
SFLC(mg/L)		
Lambda	189.7 (2-2580)	3454 (1.4-26702)
Kappa	186.1 (1.5-5318)	313.4 (0.1-6810)
IVS (mm)	11 (10-11)	11 (10-12)
NT-proBNP (ng/L)	273.1 (203.6-439.3)	381.4 (263.7-549.4)
Tent	21 (17-26)	9.5(9.5-12)
Baseline creatinine (µmol/l)	123.7 (40-979)	90.2(34-476)
eGFR (mls/min)	72.6 (10-100)	79.0 (15-90)
Albumin (g/l)	32.9 (19-53)	33.6 (17-46)

Proteinuria (g/24hr)	5.4 (0.1-23.2)	4.5 (0.05-14.8)
Bilirubin (µmol/l)	5 (4-5)	6 (6-6)
ALP (IU/l)	80.5 (74-92.4)	79968.4-86.6)
6 min walk test (m)	457.9 (92-656)	470.1 (141-697)
PFS (months)	42	50
OS (months)	Not reached	103
TTNT (months)	45	68

GI= gastrointestinal; NS= nervous system; IVS= left ventricular septal thickness; dFLC= difference in serum free light chains; NT-proBNP = N-terminal B natriuretic peptide; Tent= high sensitivity cardiac troponin; eGFR= estimated glomerular filtration rate; ALP= alkaline phosphatase; PFS= progression free survival; OS= overall survival; TTNT= time to next treatment; CI= confidence interval.

ii) Haematological response

Haematological response was assessed at six months post ASCT or bortezomib treatment and is outlined in Table 6.3. Haematological response was evaluable in 86% (n=117/136, ASCT n=64, bortezomib n=53). An overall haematological response, defined as a partial response or better, was achieved in 90.6% (n=58/64) of ASCT vs. 92.5% (n=49/53) of patients treated with bortezomib. A complete haematological response (CR) was achieved in 43.8% (n=28/64) vs. 30.2% (n=16/53) of patients treated with ASCT versus bortezomib alone.

Table 6.3: a comparison of haematological and organ responses for patients treated with ASCT and bortezomib

Response	All (n=136)	Bortezomib n=68 n(%)	ASCT n=68 n (%)
Haematological response	(n=117)	(n=53)	(n=64)
Complete response	44 (37.6)	16 (30.2)	28 (43.8)
Very good partial response	33 (28.2)	19 (35.8)	14 (21.9)
Partial response	30 (25.6)	14 (26.4)	16 (25)
No response	8 (6.8)	4 (7.5)	4 (6.3)
Progressive disease	2 (1.7)	0 (0)	2 (3.1)
Cardiac response (evaluable n=23)	(n=23)	(n=13)	(n=10)
Response	14 (60.9)	7 (53.8)	7 (70)
No response/progression	9 (39.1)	6 (46.2)	3 (30)
Renal response (evaluable n=76)	(n=76)	(n=37)	(n=39)
Response	38 (50)	9 (24.3)	29 (74.3)
No response/progression	38 (50)	28 (75.7)	10 (25.6)
Liver response (evaluable n= 27)	(n=27)	(n=9)	(n=28)
Response	8 (29.6)	2 (22.2)	6 (21.4)
No response/progression	19 (70.4)	7 (77.8)	12 (42.9)

iii) Organ response

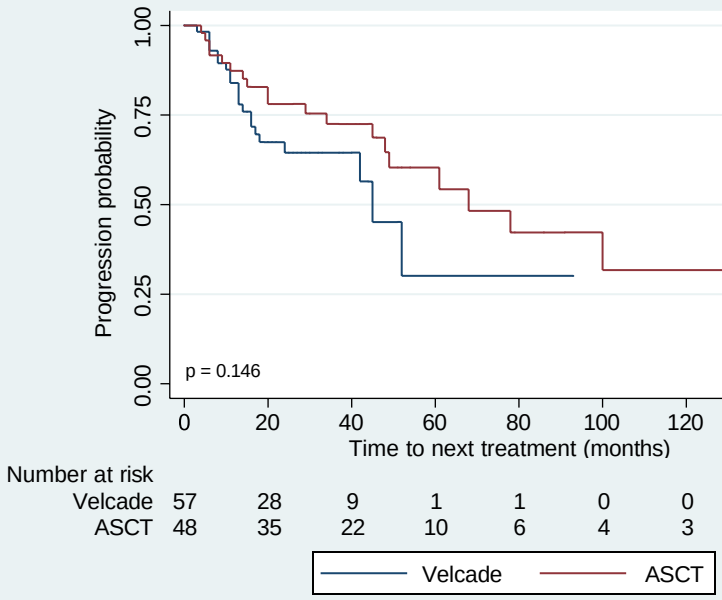
Organ response was calculated at 12 months and is outlined in Table 3. A cardiac response was evaluable in 62% (n=23/37) of patients with cardiac involvement (5 patients had died and the remaining 9 had missing NT-proBNP readings). A cardiac response was seen in 70% vs. 54% of ASCT and bortezomib patients, respectively. A renal response was evaluable in 78% (n=76/97) of patients with renal

involvement (5 patients had died and 16 patients had missing values). A renal response was seen in 74% vs. 24% of patients in the ASCT and bortezomib groups respectively. A total of 31 patients had liver involvement and 87% (n=27/31) were evaluable (three patients had missing liver function tests and one patient had died). Of these 27 patients, 21% vs 22% of patients in the ACST compared to bortezomib group had a liver response at 12 months post treatment.

iv) Progression free survival and time to next treatment

A total of 31 patients relapsed or died during the follow-up period (ASCT n=20), bortezomib (n=11). The median time to haematological progression (PFS) was 50 months vs. 42 months in the ASCT treated versus bortezomib treated groups respectively (P=0.058, HR- 0.614, CI- 0.37-1.02) (Figure 6.2).

Figure 6.2: Progression free survival in patients with AL amyloidosis treated with autologous stem cell transplant compared with bortezomib alone

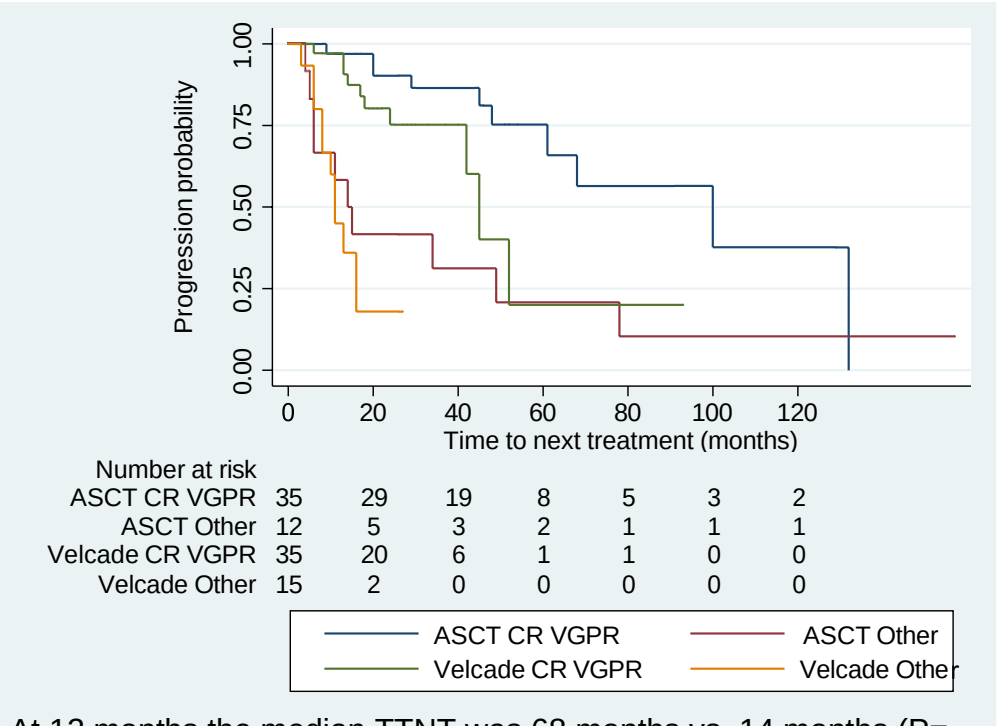


This was replicated at 12 months and 48 months post treatment (P=0.058, HR- 0.614, CI- 0.37-1.02). A landmark analysis at 12 months and 48 months also found no significant difference in the PFS at 12- and 48-months post treatment (P=0.064, HR-0.60, CI-0.35-1.03). The median time to next treatment, defined as the time from first treatment to the initiation of the next line of therapy was 68 months vs. 45 months (P=0.145, HR-0.61, CI-0.31-1.19) for ASCT versus bortezomib groups, respectively. No significant difference in the TTNT was seen at 12 months (P= 0.309, HR-0.74, CI-0.42-1.32) or 48 months (P=0.330, HR- 0.74, CI- 0.40-1.36).

Haematological response at 6 months was a highly significant predictor of both PFS and TTNT. The PFS was significantly shorter

for patients who achieved a partial (PR) or no response to treatment, compared to patients who achieved a complete (CR) or very good partial response (VGPR) in the ASCT (17 months vs. 66 months, P=0.002, HR-3.23, CI-1.56-6.67) and bortezomib group (11 months vs. 66 months, P <0.0001, HR-7.72, CI- 3.48-17.10). The PFS for patients who achieved a CR/VGPR to ASCT and bortezomib treatment was not significantly different (P=0.409, HR-1.35, CI-0.66-2.77). The TTNT was also influenced by haematological response (Figure 6.3).

Figure 6.3 Time to next treatment in patients with AL amyloidosis treated with autologous stem cell transplant compared with bortezomib alone, stratified by haematological response (complete response/very good partial response versus other response).



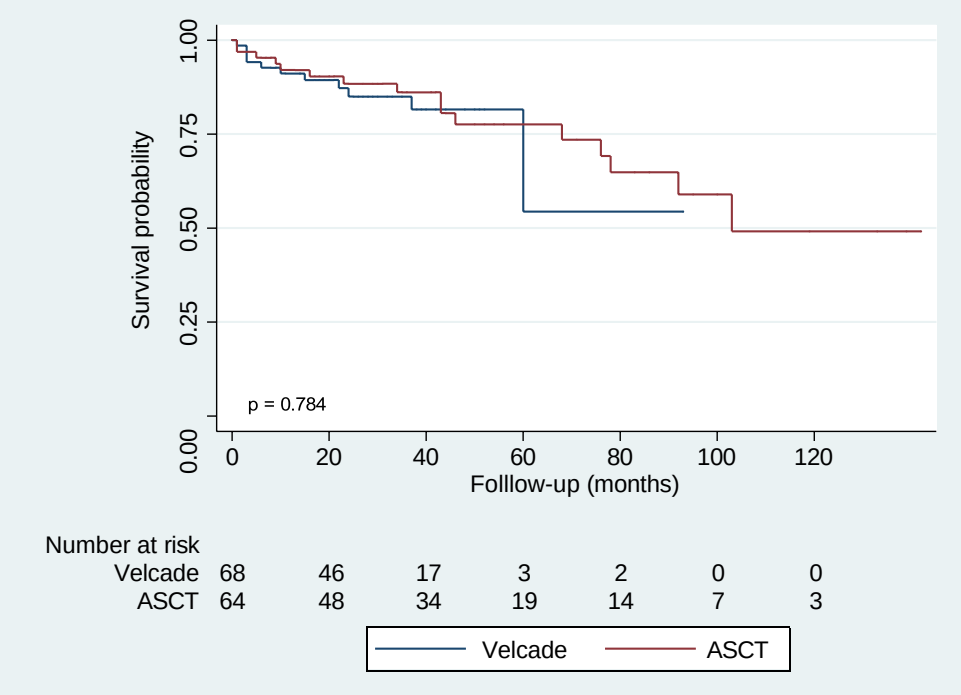
At 12 months the median TTNT was 68 months vs. 14 months (P= 0.001, HR-4.32, CI- 1.87-10.0) for ASCT patients who achieved a CR/VGPR compared with those patients who achieved a PR/ no

response. The TTNT was significantly shorter for bortezomib treated patients who achieved a PR/no response (11 months vs. 68 months, P <0.0001, HR- 8.83, CI- 3.97-19.65), but not for those who achieved a CR/VGPR to treatment (45 months vs. 68, P=0.30, HR-1.51, CI- 0.69-3.27) when compared to ASCT patients who achieved a CR/ VGPR. At 48 months the median TTNT was significantly longer for both ASCT patients (100 months vs 15 months (P= 0.002, HR-4.01, CI- 1.69-9.83) and bortezomib patients (100 months vs.11 months (P <0.0001, HR- 9.76, CI- 4.05-23.50) for patients who achieved a CR/VGPR to ASCT compared to no response to ASCT or bortezomib treatment. The TTNT was not significantly different between ASCT and bortezomib patients who achieved a CR/VGPR to treatment (100 vs 45 months, P=0.196, HR-1.69, CI- 0.76-3.71).

Overall survival

The median follow-up for the entire cohort was truncated at 120 months owing to the small number of events after this time point. In this time there were 31 deaths (ASCT n=20, bortezomib n=11). Six patients had died within 100 days of return of their stem cells in the ASCT group (TRM 8.8%, n=6/68). No deaths were recorded for patients within 100 days of receiving their first dose of bortezomib. The median OS, defined as the time from initiation of therapy to death or last follow up was 103 months vs not reached for ASCT and bortezomib groups (Figure 6.4).

Figure 6.4 Overall survival in patients with AL amyloidosis treated with autologous stem cell transplant compared with bortezomib alone



There was no significant difference in OS post treatment at 12 months (P= 0.839, HR- 0.89, CI- 0.27-2.87) or 48 months (P=0.908, HR-0.95, CI- 0.411-2.20). To overcome the possible impact of early mortality on outcome, landmark analyses were also performed at 12 and 48 months, excluding those patients who died within 6 months of starting treatment. No significant difference in OS was seen at 12 months (P=0.957, HR- 1.055, CI- 0.15-7.49) or 48 months (P=0.938, HR- 1.04, CI- 0.37-2.92). We then went on to analyse OS stratified by treatment type and haematological response. Significance was assessed by comparing the median survival against patients who achieved a CR/VGPR to ASCT. Patients who achieved a PR/no response to either ASCT (78 months, P=0.053, HR-2.18, CI- 0.99-

7.50) or bortezomib therapy (P=0.296, HR-2.03, CI-0.54-7.69) had no significant increase in their OS compared with ASCT CR/VGPR patients.

6.4 Discussion

This study demonstrates no survival advantage of ASCT compared with standard bortezomib therapy with comparable overall and progression free survival. ASCT is associated with deep haematological and organ responses rates, but without a significant improvement in the time to next treatment. Patients who achieve a partial or no response to ASCT have inferior outcomes when compared to patients who achieve a deep clonal response to bortezomib treatment.

The decision of ASCT over standard chemotherapy for the initial treatment of patients with AL amyloidosis has always been complex. Historically, the challenge was to balance the benefit of haematological and organ responses achievable with ASCT against the high transplant-related-mortality (TRM). Strict selection criteria were developed to aid with this decision process resulting in younger, fitter patients with minimal organ involvement and lower risk disease proceeding to transplant. Although this has arguably made ASCT an increasingly safe practice, a comparison of ASCT and standard chemotherapy becomes fraught with difficulty of selection bias. A

study by Dispenzieri et.al (2001) clearly highlights this issue. (137) A cohort of 229 transplant eligible patients were instead treated with standard chemotherapy. The 42-month median survival reported in this study was far longer than the median survival expected at that time with standard chemotherapy treatment of 12-18 months, and was comparable to outcomes with ASCT. The authors concluded that transplant eligibility per se is a favorable prognostic factor and urged for a direct comparison of ASCT with standard chemotherapy in a randomised trial setting. The IFM study of 2007(135) addressed this, but the inferior outcomes associated with ASCT were attributed to the high TRM. Since then the TRM for patients with AL amyloidosis has dramatically reduced. A recent analysis performed by our own centre reports a TRM of 1.1% for patients treated in the UK from 2013-2018 (Sharpley et.al, 2019). Although ASCT is an increasingly safe option, the dilemma now is that there is also an ever-expanding selection of highly efficacious chemotherapeutic agents which are also well-tolerated with minimal treatment related toxicity. This makes the decision of ASCT or novel agents increasingly difficult with an urgent need to re-assess the survival outcomes for patients in the modern treatment era.

This current data attempts to address this issue, but it should be noted that the numbers in each group after propensity scoring matching were small (n=68). The OS and PFS of patients with AL amyloidosis have markedly improved since the initial studies looking

at transplant versus conventional chemotherapy (Table 1).

But the notable finding is how comparable the outcomes between the treatment types. The median OS was not reached for bortezomib patients, compared to 103 months for ASCT, and whilst there was a trend for improved PFS/ TTNT with ASCT, this did not reach significance. The other striking finding was the marked importance of haematological response on outcomes, which appeared to be a stronger predictor of outcome than the choice of treatment. The importance of haematological response in AL amyloidosis is already well described, both in the transplant and non-transplant setting. (138, 139) Some would argue that this strengthens the argument for ASCT, given that a greater proportion of patients achieved a CR with ASCT compared with bortezomib treatment (43.8% vs 30.2%). However, to dispute this, the new and novel finding of this study is that those patients who did not achieve a deep clonal response to ASCT did significantly worse across all outcomes when compared to patients who achieved a CR/VGPR with bortezomib treatment. And this is not an insubstantial risk. Although 62% of patients achieved a CR/VGPR to ASCT, a further 32% of patients achieved only PR or minimal/no response to treatment. For these patients it could be argued that bortezomib would have been a better choice of treatment. An alternative way of looking at things is to reserve ASCT for those patients likely to achieve a deep clonal response to treatment, although at present we do not have the knowledge to predict which patients this will be.

This study is not without its limitations. This is a retrospective case-matched analysis. Incomplete data was available for both the plasma cell percentage at diagnosis and also full details of the dose of the transplant conditioning regimen. As a result, both variables could not be used in the matching process. Patients were matched using a propensity scoring statistical approach using all variables which may clinically impact survival, in addition to all variables found to significantly impact survival at the 5% level on univariable analysis. Despite this extensive effort to match patients, there are differences between the cohorts. The median time from diagnosis to treatment was longer for ASCT compared to bortezomib treated patients (7 months vs. 2 months). The bortezomib patients were also treated at a slightly later time period, from 2007 onwards, compared with the ASCT patients who were treated from 2001 onwards.

Despite these limitations, the comparable survival outcomes outlined in this paper should not be ignored, particularly as the findings have the potential to completely change clinical practice. It is therefore of paramount importance that these findings are confirmed in a larger cohort of patients and, if possible, in a prospective study. If confirmed we suggest that ASCT is reserved for patients where a deep haematological and organ response is both desirable and likely to be achievable, given the importance of clonal response on outcomes. Future efforts must now focus on identifying those patients who will likely achieve a VGPR or CR with ASCT, as it is

only these patients who should be offered an ASCT. Given that patients with t(11:14) are known to be highly sensitive to melphalan treatment,(140) we suggest that future studies should include cytogenetic analyses at baseline to assess if those patients with t(11:14) are those who are likely to achieve a CR with ASCT and who benefit most from this treatment option.

In conclusion this case-control study demonstrates no difference in survival outcomes for patients with AL amyloidosis treated with ASCT compared with standard bortezomib therapy. We must acknowledge that deep haematological and organ responses can be achieved with transplant, and also the limitations of this case-matched study detailed above, those patients who do not achieve an adequate clonal response to treatment have inferior outcomes when compared to patients who achieve a haematological response with standard bortezomib therapy. Better genetic, or other biomarkers, are required to identify patients who are likely to achieve a deep haematological response to ASCT. We urge clinicians to carefully consider the choice of first line therapy for patients with AL amyloidosis and remember that ASCT is not the only way.

Chapter Seven

Real world outcomes of AL amyloidosis patients treated with pomalidomide

This chapter is written in the context of my publication: Real World outcomes of pomalidomide for treatment of relapsed light chain **amyloidosis.** Sharpley FA, Manwani R, Mahmood S, Sachchithanantham S, Lachmann HJ, Gillmore JD, Whelan CJ, Hawkins PN, Wechalekar AD, Br J Haematol. 2018 Nov;183(4):557-563. doi: 10.1111/bjh.15541. Epub 2018 Aug 10. PMID: 30095161.

Key points:

- Pomalidomide has activity in relapsed AL amyloidosis but responses are rapid, but less durable in the real-world setting.
- Optimal responses are seen at three months but with an increasing number of non-responders at six months and early responses may be predictive of a sustained overall response.

7.1 Introduction

Systemic AL amyloidosis is a plasma cell disorder characterised by

the deposition of monoclonal immunoglobulin light chains in the form of amyloid fibrils leading to progressive organ dysfunction. Most patients present with advanced organ involvement with a poor overall

survival. The survival of patients with systemic light chain (AL) amyloidosis has improved over the last decade, with a 4 year overall survival (OS) of 54% (2010-2014) compared to 31% (2000-2004).(115) This improvement is largely a consequence of the introduction of effective, novel treatment agents.(44) More patients are surviving beyond first line treatment reflected by a reduction in six month mortality (37% in 2000-2004, to 24% 2010-2014).(115) The disease course in AL amyloidosis now more closely resembles that of multiple myeloma, characterised by remission and subsequent relapse; hence there is a need for alternative effective lines of therapy at each relapse.

Since AL amyloidosis is characterised by significant organ dysfunction, treatment must not only be effective in terms of providing a deep and rapid clonal haematological response, but also be minimally toxic to prevent any worsening of organ function. Most patients are treated with a proteasome inhibitor-based treatment in the front line setting, and a recent phase III trial has shown clear superiority of this approach over alkylator based treatment.(141) However, there is no standardised pathway for the treatment of relapsed disease. The immunomodulatory drugs (thalidomide, lenalidomide or pomalidomide) have a role in the treatment of patients with AL amyloidosis who relapse after front line treatment. Single agent thalidomide has poor tolerance and has limited efficacy.(142) Thalidomide combined with cyclophosphamide or melphalan has

reasonable activity but toxicity remains high.(143) Lenalidomide has an improved toxicity profile and is better tolerated when used at doses of 15mg per day, with overall haematological response rates ranging from 41-67%, and is widely used as a second line agent in combination with dexamethasone. (144, 145)

Pomalidomide is a next generation immunomodulatory agent that is licensed for the treatment of myeloma patients who have relapsed after treatment with lenalidomide. Pomalidomide has been reported in AL amyloidosis in three early phase trials with much better tolerance then lenalidomide and thalidomide.(1-3) Experience of this drug outside of a trial setting is however limited.

We describe the outcome of 29 patients with systemic AL amyloidosis, treated at the UK-National amyloidosis centre (NAC), with a pomalidomide based regimen.

7.2 Methods

All patients treated with pomalidomide between 2009-2017 were identified from the database of UK-NAC. Six patients were excluded as pomalidomide was initiated prior to assessment at the NAC, or the patients were lost to follow-up, leaving 29 patients eligible for analysis. Diagnosis of amyloidosis was confirmed by demonstration of characteristic birefringence under cross polarized light, with Congo-red staining, on a tissue biopsy and AL typing was confirmed by

immunohistochemistry with specific antibodies or by mass spectrometry. All patients had detailed baseline assessment for organ function, imaging and biomarker assessments. The starting dose of pomalidomide was 4mg daily (days 1-21 in a 28 day cycle) with weekly dexamethasone 20-40 mg. Monthly data was collected on treatment, toxicity and clonal response. Organ involvement was defined according to the international amyloidosis consensus criteria.(50) Haematological and organ responses were defined according to the international amyloidosis consensus criteria. (95) Organ responses were assessed from the time of starting pomalidomide to the end of therapy.(50, 146) The primary outcomes were haematological responses (HR) and overall survival (OS) following pomalidomide treatment. Overall survival was defined as time in months from start of pomalidomide treatment to death from any cause. Secondary outcomes included: progression free survival (PFS), calculated from start of pomalidomide therapy to haematological progression, or need for second line treatment, or death. Outcomes are reported on an intent to treat (ITT) basis.

Statistical analysis was performed using SPSS version 21. Approval for analysis and publication was obtained from the institutional review board at the University College London, and written consent was obtained from all patients in accordance with the Declaration of Helsinki. Survival outcomes were analyzed using the Kaplan-Meier

method with comparisons done using the log rank test. All p-values were two sided with a significance level of < 0.05.

7.3 Results

A total of 29 patients were included in this study. The patient baseline characteristics are listed in table 7.1. The median number of organs involved was 3 (range 1-6) with renal, cardiac and liver involvement in 65.5%, 69.0% and 20.7% of patients respectively. All patients had relapsed disease. The median of lines of prior treatment was 4 (range 1-7). Twenty-six (90%) patients had received prior bortezomib and 24 (83%) and 10 (35%) patients had received prior lenalidomide and thalidomide respectively. Seven percent of patients were refractory to bortezomib, 10% were refractory to lenalidomide, and 3% to both therapies. The standard dose of pomalidomide was 4mg daily, with 20mg of dexamethasone given weekly. In six patients pomalidomide was started at a lower dose, (3 patients - 3mg, 2 patients - 2mg and 1 patient - 1mg).

Table 7.1: Baseline patient characteristics of patients treated with pomalidomide

	Patients n(%)/median(range)
Median age, years	65 (41-85)
Organ involvement	3 (1-6)
Cardiac	20 (69.0)
Renal	19 (65.5)
Liver	6 (20.7)
PNS	7 (24.1)
ANS	4 (13.8)
Soft tissue	9 (31.0)
Other	10 (34.5)
Median baseline:	
Creatinine (µmol/L)	100 µmol/L
NT-pro-BNP	786 ng/L
Albumin	36 g/L
Mayo Stage at Presentation,	
I	7 (31.8)
II	9(40.9)
IIIa	6 (27.3)
IIIb	0
Missing values	7 (24.1)
Prior treatment, median no. lines (range) and included:	4 (1-7)
Lenalidomide, n (%)	24(82.8)
Bortezomib	26 (89.7)
Melphalan	12 (41.1)
Thalidomide	10 (34.5)
Other	7 (24.1)
Refractory to: n(%)	
Velcade	2(6.9)
Lenalidomide	3(10.3)
Both	1(3.4)
Duration of pomalidomide	
Months	5.0 (1-29)
Median no. of cycles	4 (1-24)

PNS= peripheral nervous system ; ANS= autonomic nervous system ; NT-pro-BNP= N-terminal pro-brain natriuretic peptide.

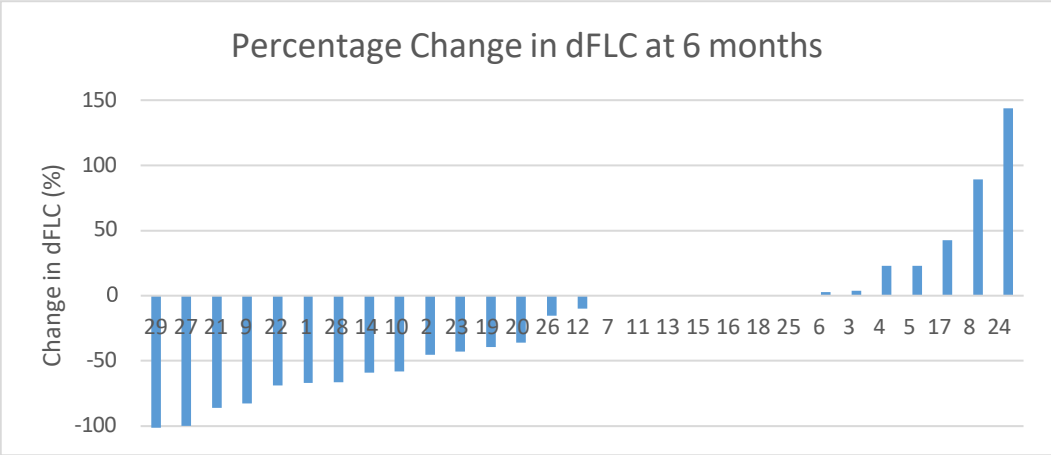
The reasons for dose reduction were: started at a low dose due to frailty and pre-existing cytopenia. The median number of cycles of pomalidomide was 4 (range 1-24) and median duration on pomalidomide was 5 months (range 1-29). Median duration of

treatment was 7 months (range 2-25) for non-responders (stable or progressive disease), and 4 months (range 3-29) for responders (partial response or better). The median NT-BNP (N-terminal pro-brain natriuretic peptide) increased in 75% of the patients on pomalidomide (from a median of 7800 ng/L (range 144-77585 ng/L) to 14690 ng/L (range 447-155161 ng/L)) at a median of 4 months of pomalidomide therapy.

Haematological responses were rapid with one patient achieving a CR and eight patients achieving a VGPR by end of one cycle. By the end of 3 cycles of treatment the haematologic responses were: CR- nil, VGPR 10 (34.5%), PR 9 (31.0%), stable or progressive disease 7(24.1%). Three patients were unevaluable owing to missing light chain measurements. The median time to best response was 3 months (range 1-6). The final response assessment was done at end of six months (missing data on one patient). On an ITT basis (n=28) at six months, no patients were in a CR, 11 (39%) had achieved a VGPR, 2 (7%) had a partial response and the remaining patients had stable or progressive disease (i.e. non-responders - 53%) (see figure 7.1). However, of the patients who had achieved a VGPR at 3 months, only 2 patients had progressed by six months. Of the patients not achieving a VGPR or better by 3 months, only one additional patient achieved a VGPR at 6 months. There was no impact of prior bortezomib or lenalidomide exposure on depth of response.

Figure 7.1 : Percent Change in the difference in free light chains

(dFLC) at 6 months: CR-nil, VGPR-11 (37.9%), PR-2 (6.9%), NR-8 (27.6%), PD- 7 (24.1%), missing-1 (3.4%)



dFLC= difference in serum free light chain

Since, cardiac response was assessed by NT-proBNP values, to minimise the impact of the increase in NT-proBNP with pomalidomide treatment, we evaluated organ responses at six months and also at the end of pomalidomide treatment. Of the 20 patients with cardiac involvement, 13 patients were evaluable at six months (the remaining 4 patients with NT-proBNP <650 ng/L and 3 others with missing NT-proBNP values). Of these patients, 38% (5/13) had a cardiac response, 46% (6/13) cardiac progression, and 15% (2/12) were non-responders. At the end of pomalidomide treatment 14 patients were evaluable: 43% (6/14) with a cardiac response, 29% (4/14) with cardiac progression and 29% patients (4/14) were non-responders.

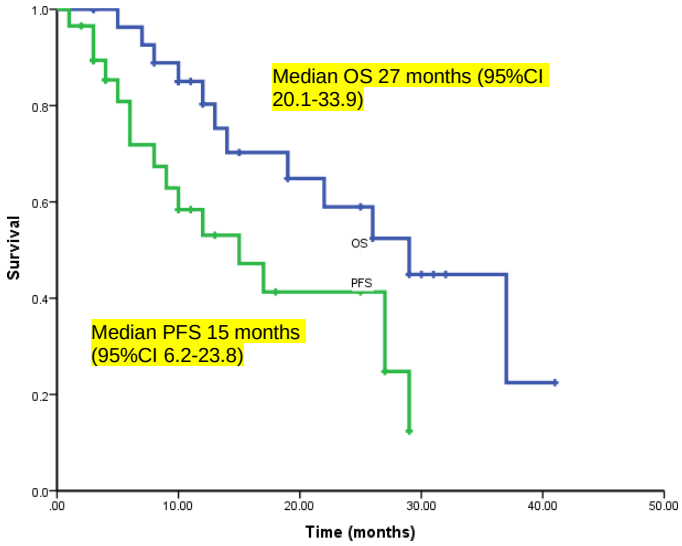
Only one additional patient therefore achieved a cardiac response after stopping pomalidomide and so there was only a small actual bias

introduced by the increase in NT-proBNP on response assessment. The median time to reach a cardiac response was 7 months (3-9 months).

Of the 19 patients with renal involvement, four patients were established on dialysis prior to pomalidomide and one patient died before repeat creatinine readings were taken leaving 14 patients eligible for analysis. Seven patients had an increase of 25% of their creatinine during pomalidomide therapy, but only one patient went on to require renal replacement therapy. For the remaining six patients, two patients' renal function has continued to deteriorate after stopping pomalidomide therapy (but they remain dialysis independent), one patient's renal function has improved, two patients have not had repeat creatinine readings (one due to death and the second due to no follow-up since stopping pomalidomide). Seven patients' creatinine readings remained stable on pomalidomide treatment, and no patients' creatinine readings improved. Renal response was assessed by proteinuria measurements at 6 months. Renal progression was seen in 33% (3/9) and a renal response was seen in 44% (4/9) and no response in 22% (2/9) patients. All three patients with renal progression were non-responders, i.e. had stable or progressive disease. This suggests that these were true renal amyloid progression events, rather than pomalidomide induced.

With a median follow-up of 13 months (2-37 months), there were 12 deaths. The median overall survival from start of pomalidomide was 27 months (95% CI 20.1-33.9 months) (Figure 7.2). The overall survival (OS) for patients achieving response at six months was: very good partial response (VGPR) or better 37 months, partial response (PR) 27 months, non-responders 15 months, progressive disease 19 months. The median progression free survival was 15 months, (95%CI 6.2-23.8 months) (Figure 7.2).

Figure 7.2 Overall Survival (OS) of 27 months and progression free survival (PFS), of 15 months



The most common adverse events were: non-neutropenic infection (56%), lethargy (56%), sensory neuropathy (44%), neutropenia (33%), pain (33%), constipation (22%), diarrhoea (22%), fluid overload (22%), hypotension (11%), mucositis (11%), peripheral motor neuropathy (11%), rash (11%), and somnolence (11%). The highest CTCAE grade was 3 and the adverse events with this grade were: non- neutropenic infection (33%), fatigue (33%), neutropenia (22%), sensory neuropathy (22%), fatigue (11%), fluid overload (11%), renal impairment (11%). Nineteen patients have stopped pomalidomide treatment, 1 has died and 9 patients remain on ongoing therapy. The reason for discontinuing therapy was available in 17/19 (89%) of patients. Six patients (35.2%) stopped pomalidomide due to a planned clinical decision, since the patient had reached an adequate haematologic response. Seven (41.1%) patients discontinued due to adverse events – one patient each due to: fatigue, worsening peripheral sensory neuropathy, renal impairment, worsening orthostatic hypotension and frailty, respectively, and in two cases due to patient preference. Four patients (23.5%) discontinued pomalidomide due to stable or progressive disease and only two patients went on to receive a further line of therapy after pomalidomide, one with carfilzomib and the other with thalidomide based therapy.

7.4 Discussion

This data demonstrates that pomalidomide has activity in patients with AL amyloidosis at relapse with patients achieving a relatively rapid response by 3 months. Some patients, even in this heavily pre-treated patient population, achieve deep clonal responses of VGPR or better, however this real-world data suggests that despite encouraging early responses longer term benefits appear much less. A significant proportion of patients die or discontinue therapy, and there is a lack of persisting response with 52% having no response, died or progressed by 6 months.

There have been three previous phase 2 trials conducted with pomalidomide in the setting of AL amyloid. Table 7.2 summarises the previous trials and outcomes.

Table 7.2: A comparison of the three previous phase 2 trials of pomalidomide in AL amyloidosis, the Mayo group (2012)(1) the Boston group (2016)(2) the Italian group(3) and the data presented here from the NAC (National Amyloidosis Centre).

	Mayo (2012) n(%)	**Boston (2016) n(%)**	**Italian (2017) n(%)**	**NAC (2018) n(%)**
Patient no.	33	27	28	29
Prior regimens Alkylator IMIDs PI ASCT	 30 (91) 7(21) 14 (42) 16 (48)	 / 13 (48) 21 (78) 16 (59)	 21(75) melphalan, 19 (88)cyclophosphamide 11 (39) 27 (96) 6(21)	 12 (41.1) 26(89.7) 27 (90) 5 (17)
Organs involved Heart Kidney Liver	 27 (82) 12 (36) 1 (3)	 18 (67) 14 (52) /	 22 (79) 11 (39) 1 (4)	 20 (69) 19 (66) 6 (21)
Time from diagnosis to enrolment (months)	37	27	16	
Treatment Pomalidomide dose (mg) Dexamethasone dose (mg) Duration of treatment (median no. of cycles)	 2 40 weekly	 2(d1-28), 3(d1-21) MTD 4mg 20 weekly 6 (0-18)	 MTD 4mg 20 weekly 6(1-30)	 N/A N/A
Overall haematological response (6 months) VGPR/CR	16(48) 6(18)	12 (50)	17(61) 7(25)	13(46)
Organ response rates Cardiac Renal	 4(15) 2(17)	 / 1(7)	 / 2(17)	
Overall Survival (months)	27.9	Not reached	26	27
Progression free survival (months)	14.1	17.8	16	15
Severe myelosuppression	15 (45)	7 (25.9)	2(7.1)	1 (3)

Treatment discontinued Due to AEs or patient refusal Due to PD or death	27(82) 11 (33) 15 (45)	24(89) 5(29) 11 (41)	26(93) 11 (39) 14 (50)	19 (66) 7 (39) 4 (24)

IMID= immunomodulatory drug (includes thalidomide, lenalidomide); PI= proteasome inhibitor; ASCT= autologous stem cell transplant; AE= adverse event; PD= progressive disease, MDT= maximum tolerated dose; VGPR= very good partial response; CR= complete response; NAC= National Amyloidosis Centre.

The overall survival of patients treated with pomalidomide is remarkably similar in all previous studies, (OS of 26-28 months), and the outcomes of this current cohort are comparable with an OS of 27 months. Likewise, a PFS of 15 months in this current cohort is comparable to the previously reported PFS of 14-17.8 months. In our current cohort, the overall response rate was similar to the Italian cohort at 3 months (66%). The Italian group however report best response at 7 cycles, which is very different from our cohort where median time to best response was 3 months. In our cohort, only one patient who had not achieved a VGPR by 3 months improved depth of response and, indeed, two patients with VGPR at 3 months had progressed by 6 months. This suggests that early response predicts the longer term response and that prior therapy may affect the durability of haematologic responses. Interestingly this is similar to our previous data using CTDa where we found very few responses beyond three months and this resulted in a change in clinical practice at our centre, reviewing therapy at 3 months to add/switch to an alternative agent.(72) It appears intriguing that Pomalidomide, which has more

structural similarity to thalidomide than lenalidomide, appears to show a similar pattern.

Two factors may be limiting the duration of response in our cohort compared to the previous studies: the majority of our patients had prior IMiD based treatment; also, the standard practice in UK is for patients to receive a fixed duration of treatment. A quarter of patients in the current series had planned discontinuation of treatment after achieving a haematologic response. Since almost all studies with pomalidomide in AL and in myeloma have used continuous therapy, there is limited data on progression after stopping pomalidomide. Based on data from previous AL studies with other regimens, (72) we know that patients can remain in a stable haematologic response even after discontinuing therapy – indeed in the current cohort of the 6 patients who stopped therapy in a planned manner – 2 relapsed and 4 are still in remission. This suggests that in some patients after achieving a deep response, where tolerance may be a problem, discontinuation of pomalidomide could considered.

The toxicity profile of pomalidomide when used in myeloma is favorable, in a recent pooled analysis of 1088 myeloma patients only 9.7% of patients had to discontinue pomalidomide therapy, with myelosuppression most commonly reported.(143) In AL amyloidosis, this is remarkably different with discontinuation rates of 60-93% in the

previous studies. In our cohort, 38.9% were unable to tolerate therapy with side effects ranging from fatigue to worsening of neuropathy and orthostatic hypotension – consistent with previously reported data. A limiting feature of this series is the limitation of a retrospective series in capturing true adverse event data– the reported number is likely to be an under-representation of the true toxicity of pomalidomide.

In conclusion, pomalidomide combined with dexamethasone is a useful treatment option for patients with AL amyloidosis with relapsed refractory clonal disease. A significant proportion of patients achieve good haematologic responses, however responses are not as deep nor as durable in the real-world setting. Responses are rapid and early responses appear to define longer term outcomes. Pomalidomide is not as well tolerated in AL amyloidosis as myeloma and careful dose titration of pomalidomide may allow more patients to remain in therapy and benefit from longer term responses. Combination studies of pomalidomide with other agents like proteasome inhibitors or Venetoclax may offer additional and deeper responses and needs future prospective studies.

Chapter Eight

Cytomegalovirus reactivation after treatment with bortezomib

This chapter is written in the context of my publication:

Cytomegalovirus reactivation after bortezomib treatment for multiple myeloma and light chain amyloidosis Sharpley FA, De-Silva D, Mahmood S, Sachchithanantham S, Ramsay I, Garcia Mingo A, Worthington S, Hughes D, Mehta A, Kyriakou C, Griffiths PD, Wechalekar AD. Eur J Haematol. 2020 Mar;104(3):230-235. doi: 10.1111/ejh.13366. Epub 2020 Jan 10. PMID: 31815313.

Key points:

- There is a substantial risk of cytomegalovirus reactivation in patients with systemic AL amyloidosis or multiple myeloma treated with bortezomib
- CMV reactivation occurred in seropositive, rather than seronegative patients suggesting reactivation/reinfection rather than primary infection
- CMV disease was not seen but pre-emptive anti-viral treatment was required in 36% of cases.

8.1 Introduction

Cytomegalovirus is a DNA virus of the *Herpesviridae* family. In the western world up to 90% of the population are positive for IgG to CMV. This almost ubiquitous virus is transmitted by bodily fluids which includes blood, urine, saliva, semen and breast milk and can infect epithelial, smooth muscle, blood and endothelial cells. In the immunocompetent host, primary infection is usually asymptomatic and usually occurs at a young age, but then the virus becomes dormant, establishing lifelong latency within host cells. Host T and natural killer (NK) cells are essential to controlling CMV infection and in the setting of impaired cellular immunity the virus can reactivate from latency.(147) Immunocompromised patients are at risk of symptomatic infection which can range from a febrile episode (temperature >38 degrees for at least 2 days within 4 days) with neutropenia and/ or thrombocytopenia, to fatal disease which can include: pneumonia, colitis, hepatitis, retinitis, myocarditis, central nervous system disease or pancreatitis.(148) Allogenic stem cell transplant recipients are well recognised as patients at risk of CMV disease(149) and guidelines are available for the screening, monitoring and treatment of CMV in this setting.(149) The risk of symptomatic CMV disease for haematology patients treated with standard chemotherapy or an autologous stem cell transplant (ASCT) is historically considered to be low.

Bortezomib is an inhibitor of the 26S proteasome widely used in the treatment of multiple myeloma and AL Amyloidosis.(150) Bortezomib is associated with an increased incidence of reactivation of other herpesviruses, including varicella zoster virus (VZV) and herpes simplex virus type 1 (HSV-1).(151) Bortezomib appears to reduce the lymphocyte number and alter the Th1/Th2 balance, resulting in susceptibility to infections, with significantly more viral and fungal infections in patients treated with the drug.(152).

A case of CMV reactivation in a 72 year old female with systemic AL amyloidosis treated with cyclophosphamide, bortezomib and dexamethasone prompted this prospective study. The patient presented with significant weight loss, vomiting and diarrhoea requiring prolonged hospital admission. Whilst this was initially thought to be due to the patients' autonomic dysfunction, during investigations she was found to have a CMV viral load of >1.4 million copies. Her systemic CMV infection with predominant gut symptoms was treated with ganciclovir until her CMV copies were undetectable, with complete resolution of her symptoms. As a result of this index case we prospectively measured CMV copies/ml in patients with newly diagnosed multiple myeloma or systemic AL amyloidosis treated with bortezomib based regimen.

8.2 Methods

The National Amyloidosis Centre (NAC) provides a tertiary referral service for patients with amyloidosis and related disorders in the UK. The referring local hospitals are the treating centres, and this includes the Royal Free Hospital. Consecutive, newly diagnosed patients with multiple myeloma or AL amyloidosis attending NAC and consented for bortezomib treatment at the Royal Free Hospital from August 2014 – August 2015 were included in this study. The diagnosis of multiple myeloma was made according to established International Myeloma Working Group (IMWG) criteria. (153) A diagnosis of amyloidosis was confirmed by Congo red staining of a tissue biopsy with demonstration of characteristic birefringence under cross-polarized light. The amyloid subtype was confirmed by immunohistochemistry with specific antibodies, or by mass spectrometry.(121) Hereditary amyloidosis was excluded by gene sequencing as appropriate. All patients had a detailed baseline assessment of organ function with biomarker assessments and imaging, including SAP scintigraphy, where appropriate. Organ involvement was defined according to the international amyloidosis consensus criteria.(86) All patients had protein electrophoresis, immunofixation and serum free light chains quantified. The presence of immunoparesis was defined as one or more immunoglobulins less than the lower limit of normal. All patients were treated with a bortezomib containing regimen, either as a single agent or in

combination. The choice of regimen was at the discretion of the treating clinician, based upon the patient's organ function, comorbidities and performance status.

All patients had serologic testing for CMV-specific antibodies (IgG) prior to treatment. CMV testing was performed using the ARCHITECT CMV IgG assay, which is a chemiluminescent microparticle immunoassay for the qualitative and semi-quantitative determination of IgG antibodies to CMV. Patients with >6AU/ml were deemed to be seropositive for CMV. The CMV viral load was measured by real-time polymerase chain reaction (RT-PCR) at baseline and then every two weeks from the date of their first dose of bortezomib, regardless of symptoms. Extraction of DNA from patient whole blood was performed on the QI symphony, using the QIAquick PCR purification kit, and quantification of CMV DNA using the artus CMV RGQ MDx Kit. Amplification of the DNA was performed on the Rotor-Gene Q. Positive CMV DNAemia was defined as the detection of CMV DNA in whole blood (Table 8.1).

Table 1: Cytomegalovirus infection definitions. Adapted from Ljungman *et. al(148)*

Type of infection	*Definition*
Viraemia	Isolation of CMV using cell culture assay
Antigenemia	Detection of CMV pp65 in peripheral blood leucocytes
DNAemia	Detection of CMV DNA in whole blood, isolated peripheral blood leukocytes
Primary CMV infection	Detection of CMV virus or viral proteins or nucleic acid in a seronegative patient
Recurrent CMV infection	New detection of CMV in a patient with a previously documented primary infection after no virus has been detectable after a period of at least 4 weeks of active surveillance

CMV, cytomegalovirus; pp65, phosphoprotein 65.

Statistical analysis was performed using SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) and Stata (StataCorp. 2017. Approval for analysis and publication was obtained from the institutional review board at the University College London. Written consent was obtained from all patients in accordance with the Declaration of Helsinki.

8.3 Results

A single CMV viral load greater than 7500 copies/ml was regarded as an indication for anti-viral treatment. In such cases patients were treated as per British Society of Haematology guidelines, (154) with oral valganciclovir (900mg twice a day) for 14 days, followed by a maintenance dose of valganciclovir (450mg twice a day), or IV ganciclovir (5mg/kg/day) for 14 days, continued at a maintenance dose of 5mg/kg/day, until the CMV viral load was undetectable. (148)

Baseline patient characteristics

A total of 57 patients (38 AL amyloidosis and 19 multiple myeloma) were included. The baseline patient characteristics are detailed in Table 8.2. Immunoparesis was present in 77.2% (n=44/57) of patients. CMV serology results were available in 78.9% (n=4/57) and 68.9% of patients were CMV seropositive (n=31/45, 68.9%). No patients had detectable viral copies prior to treatment. All patients were treated with a bortezomib containing regimen. The majority (92%, n= 52/57) of patients were treated with a triplet regimen and 78.9% of patients received bortezomib, cyclophosphamide and dexamethasone (VCD), (n=45/57). Four patients were treated with a doublet (7.0%, n=4/57), bortezomib and dexamethasone (VD) and one patient with a quadruplet regimen bortezomib, melphalan, thalidomide and prednisolone (VMTP) (1.8%, n=1/57).

Table 8.2: Summary patient characteristics, including those with cytomegalovirus reactivation

	All patients (%/ range), n=57	**CMV reactivated patient n=14**
Diagnosis		
AL amyloidosis	38	7
Multiple Myeloma	19	7
Gender		
Male	33	9
Female	24	5
Age, years, (median/range)	63 (33-89)	63 (37-78)
Paraprotein Isotype		
None	31	6
IgG	16	4
IgA	5	3
IgM	3	1
IgD	1	0
IgG and IgA	1	0
Paraprotein g/L (median/ range)	20.5 (1.1- 97)	38.5(3-97)
FLC ratio (range)	<0.01 - 6166.7	<0.01 - 6166.7
FLC mg/L (median/range)	1924.5 (28.1 - 16000)	867 (103 - 9250)
Immunoparesis		
Yes	44	12
No	13	2
Proteinuria		
Yes	41	10
No	13	3
Unevaluable	6	1
CMV serostatus pre-treatment		
Positive	31	12
Negative	14	0
Unevaluable	12	2
Chemotherapy regimen		
Bortezomib +steroids (VD)	3	0
Bortezomib+steroids:		
+ Alkylator (VCD/VMP/ VC/ VCP)	48	12
+ IMiD (VTD/ VRD/ VMTP/ VCTD)	5	1
+ Anthracycline (PAD)	1	1

CMV, cytomegalovirus; AL, light chain amyloidosis; Ig, immunoglobulin; FLC, free light chain; VCD, velcade, cyclophosphamide, dexamethasone; VD, velcade, dexamethasone; VRD, velcade, lenalidomide, dexamethasone; VTD, velcade, thalidomide, dexamethasone; VC, velcade, cyclophosphamide; VCTD, velcade, cyclophosphamide, thalidomide, dexamethasone; VCP, velcade, cyclophosphamide, prednisolone; VMTP, velcade, melphalan, thalidomide, prednisolone; PAD, Bortezomib, doxorubicin, dexamethasone.

CMV reactivation

CMV reactivation was detected during bortezomib treatment in 25% (n=14/57) of patients. The patient and treatment details of these 14 patients are detailed in Table 1. An equal number of myeloma (50%, n=7/14,) as AL amyloidosis (N=7/14, 50 %) patients has CMV reactivation, however a greater proportion of myeloma versus AL amyloidosis patients developed detectable CMV copies during treatment (37%, n= 7/19 versus 18%, n=7/38, respectively). Those with CMV DNAemia had proteinuria in 77% of cases (n=10/13 (1 patient unevaluable),) and immunoparesis in 86% (n=12/14). The patients with DNAemia were seropositive in 86% of cases (n=12/14); the remaining two patients had missing baseline CMV serology. No CMV DNAemia was seen in the seronegative group.

The majority of patients developed detectable viral copies within the first 48 days of bortezomib based treatment (23%, n=3/14, during cycle 1; 50%, n= 7/14 during cycle 2). In the four remaining patients, (21%, n=3/14) developed detectable copies at the end of cycle 3 and one (7%, n=1/14) during cycle 4 (Fig. 1).

CMV treatment details

In the 14 patients who had detectable CMV copies, five (36%, n=5/14) reached the threshold to require pre-emptive anti-viral CMV anti-viral treatment. No patients were deemed to have symptoms suggestive of active CMV disease. No patients were admitted to

hospital due to CMV disease, but two patients with detectable viral load required admission for management of Influenza A.

8.4 Discussion

CMV reactivation or infection has the potential for serious morbidity or mortality in patients with haematological malignancies. Whilst the role of CMV infection is well characterised in the setting of allogeneic stem cell transplantation, its importance in multiple myeloma and AL amyloidosis has not been well studied. In this small prospective study, we report that nearly 40% of CMV seropositive individuals reactivate CMV during early treatment course with bortezomib. Whilst no patients developed clinical CMV disease, pre-emptive treatment was required in 36% of cases, which may have precluded clinical CMV infection. This suggests that bortezomib is associated with a substantial risk of CMV reactivation.

The risk of CMV reactivation is associated with both the degree of immunosuppression and, specifically, the degree of T-cell depletion. (155) Bortezomib is a highly efficacious proteasome inhibitor widely used to treat multiple myeloma and AL amyloidosis patients. A consequence of treatment is diminished cellular immunity, in particular the proliferation and function of CD8+ T lymphocytes and NK cells, (156) resulting in susceptibility to infections. (152) The risk of reactivation of herpesviruses, including

varicella zoster virus (VZV) and herpes simplex virus type 1 (HSV-1), is well recognised.(151) In the APEX study, bortezomib treatment was associated with a significantly higher incidence of herpes zoster compared with dexamethasone treatment (13%, n=42/331 versus 5%, n=15/332 respectively; P = 0.0002), and no other risk factors could be identified. (157) This study resulted in a change in clinical practice with the routine use of prophylactic acyclovir for the majority of patients treated with bortezomib. The exact mechanism of bortezomib induced zoster reactivation remains debatable.

CMV is also a herpesvirus, and so there is a similar theoretical risk of CMV reactivation associated with bortezomib treatment. The difficulty of diagnosing CMV reactivation, in the absence of routine monitoring, makes this risk difficult to quantify. There has been a scattering of case reports of CMV reactivation in patients treated with bortezomib based regimens. (158) An increased risk of CMV reactivation has also been reported in patients treated with bortezomib induction followed by an ASCT.(159, 160) Kim *et. al* (2012) retrospectively evaluated 104 patients with multiple myeloma treated with ASCT with an overall CMV reactivation rate of 30.8% (n=32/104), and 48.5% (n=32/66) of CMV-seropositive patients developed detectable CMV copies. Patients who received conditioning therapy with melphalan, bortezomib, dexamethasone, and thalidomide were significantly more likely to develop CMV reactivation (P = 0.015). (160) A prospective study performed by

Marchesi *et. al* (2013) of 80 patients with multiple myeloma treated with ASCT also reported a significantly higher rate of CMV reactivation in patients who received bortezomib and immunomodulatory therapy when compared to standard anthracycline based treatment (9.4% versus 1.1% p=0.019), but not in patients treated with immunomodulatory therapy alone. The study concluded that patients treated with bortezomib-based regimens were at higher risk of developing symptomatic CMV reactivation after ASCT. (161) A more recent retrospective study performed by Hasegawa *et.al* (2016) of 120 patients with multiple myeloma reported a CMV infection rate of 20% and three cases of CMV disease. This study included patients who did not proceed to ASCT, and 80% of the patients with CMV reactivation were treated with bortezomib. (162)

A comprehensive literature review performed by Marchesi *et al* (2017) of non-transplant haematology patients revealed highly-variable CMV reactivation rates ranging between 2-39%, (161) but this included a wide range of agents including bortezomib, bendamustine and rituximab. The CMV reactivation rate of 25% in this current study compares with the 20% figure, reported by Hasegawa *et al* (162) and the higher rate of CMV reactivation ~40% in CMV seropositive patients here is also in keeping with the 48.5% reported by Kim *et.al* (160), suggesting that this is a true and replicable finding.

Little is known about the risk of CMV reactivation with time/ duration of treatment. In this current study the majority of CMV reactivation events occurred during the first two cycles of bortezomib treatment. Hasegawa *et al* (2016) reported a median duration from diagnosis to CMV reactivation of 5 months (1-86 months) in CMV seropositive patients, with a significantly longer interval in CMV negative patients, where the median was 20 months (1-84 months) (P= 0.025). This suggests that CMV reactivation may be an event which occurs during the course of treatment, although further studies are required to confirm this finding.

Factors predicting CMV reactivation also remain unclear. In our study, which included patients with systemic AL amyloidosis, there appeared to be higher proportion of patients with immunoparesis (n=44, 77%) and proteinuria (n= 41, 72%). This was also reflected in the patients who developed detectable CMV copies (immunoparesis, n=12/14, 86%; proteinuria, n= 10/14, 71%). Both variables seem to be independent risk factors as there was no correlation between proteinuria and immunoparesis (Pearson Chi-square P=0.769). Other studies have reported the presence of extramedullary disease and low absolute neutrophil count as risk factors, (162) but this may well simply be a marker of extensive pre-treatment.

The current data suggests that reactivation is the major cause of CMV DNAemia since none of the seronegative patients developed

detectable CMV copies/ml and no patients developed CMV disease. Due to use of a pre-emptive treatment strategy, this study is unable to infer how many patients would have developed CMV end-organ disease without treatment. However, in the study by Hasegawa *et al* (2016), where there was no pre-emptive treatment strategy, 66% of patients developed clinically significant CMV disease. The lack of CMV disease in this study suggests one of two options: that the CMV reactivation rate is significant in patients treated with bortezomib, but that the risk of CMV disease is low, as suggested by previous guidelines (163) and/or that a pre-emptive anti-viral treatment is an effective strategy to prevent symptomatic infection.

This study is not without limitations. This was a small study with AL and myeloma patients. The baseline CMV serology was known in all but two cases; we cannot therefore fully exclude primary infection in these two cases. Patients were also pre-emptively treated, hence the true incidence of clinical CMV disease remains unclear. All patients were treated with bortezomib in combination with steroids +/- a third chemotherapeutic agent, we cannot therefore definitively prove that bortezomib resulted in CMV reactivation. Despite these limitations, this study confirms that this is a risk of CMV reactivation in patients treated with bortezomib. The clinical significance of these reactivations requires further study, particularly as more complex triplet or quadruplet regimens, with greater consequent

immunosuppression, are being used to treat patients with multiple myeloma and AL amyloidosis. Given the difficulty of clinically recognising CMV infection, the current findings also raise an important issue of CMV viral monitoring, which must be addressed in further prospective studies in larger patient cohorts. Physicians must also remain alert to the possibility of CMV infection with bortezomib treatment in patients with relevant atypical infective symptoms.

Chapter Nine

Amyloidosis diagnosed in solid-organ transplant recipients

This chapter is written in the context of my publication: Amyloidosis diagnosed in solid-organ transplant recipients Faye A Sharpley, Marianna Fontana, Janet A Gilbertson, Julian D Gillmore, Philip N Hawkins, Shameem Mahmood, Richa Manwani, Ana Martinez-Naharro, Cristina Quarta, Tamer M Rezk, Dorota Rowczenio, Sajitha Sachchithanantham, Carol J Whelan, Ashutosh D Wechalekar and Helen J Lachmann. Transplantation. 2020 Feb;104(2):415-420. doi: 10.1097/TP.0000000000002813. PMID: 32004234.

Key points:

- Amyloidosis may occur post solid organ transplant with an overall poor survival.

9.1 Introduction

Solid organ transplantation carries an increased risk of malignancy which has been attributed to the requirement for long term immunosuppression. Skin cancer is the most common malignancy, followed by post-transplant lymphoproliferative disorder (PTLD).(164) The risk of PTLD can be as high as 10% and is largely dependent on

the type of organ transplanted with the highest risk in intestinal and the lowest in renal transplants, probably reflecting the degree of immunosuppression required;(165) age and length of time post-transplant are also recognised risk factors.(166)

The World Health Organisation provides a histological classification system for PTLD.(167) Approximately 85% of cases of PTLD are B cell in origin(168), and the plasma cell neoplasms (PCN) are a rare form of monomorphic type PTLD.(169) In a large study of 202,600 solid organ transplant recipients from the United States the estimated incidence of PCN was 15.4 per 100,000 person years, which represents a 1.8 fold increase compared with the general population.(170) The majority of cases described were multiple myeloma (N=102/140), with fewer cases of plasmacytomas (N=38/140).(170) No cases of systemic AL amyloidosis were described. Nonetheless systemic AL amyloidosis is a well-recognised complication of B cell disorders and is therefore a potential complication of PTLD. There is little in the literature regarding this risk presumably reflecting the rarity of both PTLD associated PCN and AL amyloidosis. Here we report a series of 30 UK patients diagnosed with amyloidosis following a solid organ transplant. Our hypothesis was that AL amyloidosis can develop after a solid organ transplant as a rare complication of PCN- PTLD.

9.2 Methods

We searched our database of 5,112 patients seen from 1994-2018 with a diagnosis of amyloidosis for solid organ transplant. 427 cases were excluded as the diagnosis of amyloidosis preceded the transplant date. The indication for solid organ transplantation, the transplant date and the organ transplanted were recorded. In all cases amyloidosis was confirmed on biopsy material by Congo-red staining with demonstration of characteristic birefringence under cross polarized light. The amyloid fibril sub-type was established by immunohistochemistry using a panel specific antibodies or by mass spectrometry.[171],[172] Where a definitive diagnosis was not made by immunohistochemistry, genetic testing was used to exclude hereditary amyloidosis. All patients had a detailed baseline assessment including organ function, imaging with SAP scintigraphy and echocardiogram and biomarker assessments.(57) Organ involvement with amyloidosis was defined according to the international amyloidosis consensus criteria.(50) Treatment details were recorded, including transplant immunosuppression and treatment aimed at the underlying amyloidogenic condition. Hematological responses were assessed at six months and organ response at 12 months, both calculated from the date of diagnosis and defined according to the international amyloidosis consensus criteria.(50) We also gathered details about the graft survival, where data were available.

Statistical analysis was performed using SPSS version 21. Survival outcomes were analyzed using the Kaplan-Meier method. Approval for analysis and publication was obtained from the institutional review board at the University College London and written consent was obtained from all patients in accordance with the Declaration of Helsinki.

9.3 Results

Thirty patients (19 male, 11 female) were included.

i) Patient transplant characteristics

The 30 patients received solid organ transplants between 1970 and 2013. Details of the reason for organ transplantation, the organ type and the immunosuppressant taken at the time of diagnosis with amyloidosis are outlined in table 9.1. The median age at transplant was 44 years (range 10-71 years). The organ transplanted was kidney (N=25, 83.3%), liver (N=2, 6.7%), heart (N=2, 6.7%), with the final patient having a combined heart, lung and kidney transplant (N=1, 3.6%). The cause of organ failure was available in 67% of cases (20/30 patients) and are listed in table 9.1.

ii) Characteristics of amyloidosis

a. The entire cohort of patients

All 30 patients had histological confirmation of amyloid deposition. The median age at the time of diagnosis with amyloidosis was 52

years (range 33-77 years). The most frequent type of amyloidosis was light chain (AL) (N=14, 46.7%), followed by AA (N=11, 36.7%), localised AL (N=3, 10%), wild type transthyretin (wtATTR) (N=1, 3.3%) and amyloid of uncertain type (N=1, 3.3%). The median time from date of transplant to diagnosis of amyloidosis was 10.5 years (range 7 months to 36 years).

Table 9.1: Transplant characteristics (N=30)

	Number
Age at time of transplant	44 (range 10-71)
Organ transplanted and amyloid type	
Kidney (N=25)	
AA amyloid	9 (36%)
AL amyloid	12 (48%)
Localised AL amyloid	2 (8%)
ATTR amyloid	1 (4%)
Liver (N=2)	
Localised AL amyloid	1
AL amyloid	1
Heart (N=2)	
AA amyloid	2
Heart, lung and kidney (N=1)	
AL amyloid	1
Reason for organ transplant	
Kidney	
AA amyloid (N=9)	
Hypertensive nephropathy	2
'Small kidneys'	1
Mesangiocapillary glomerulonephritis	1
Reflux nephropathy	1
Unknown	4
AL amyloid (N= 12)	
Fibrillary glomerulonephritis	1
Focal segmental glomerulosclerosis	1
Adult polycystic kidney disease	1
Systemic lupus erythematosus	1
Chronic interstitial nephritis	1
Reflux nephropathy	1
Megacystic megaureter	1
IgA nephropathy	1
Unknown	4
Localised Al amyloid (N=2)	
Adult polycystic kidney disease	1
Unknown	1
ATTR amyloid (N=1)	
Diabetic nephropathy	1
Uncertain type (N=1)	
Systemic lupus erythematosus	1

Liver (N=2)	
AL amyloid	
Primary biliary cirrhosis	1
Localised AL amyloid	
Paracetamol overdose	1
Heart (N=2)	
AA amyloid	
Eosinophilic granulomatosis with polyangiitis	1
Unknown	1
Combined heart, lung, kidney (N=1)	
AL amyloid	
Cystic fibrosis and ciclosporin toxicity	1
Immunosuppression at diagnosis with amyloidosis	
Mycophenolate	3
Tacrolimus	3
Ciclosporin	2
Prednisolone	2
Combination (2+ agents)	3
Missing	17
Reason for renal graft failure (N=11)	
Amyloidosis	4
Renovascular disease	1
Unknown	4
Median graft survival (months)	
From time of transplant	185 (96-269)
From diagnosis with amyloidosis	2 (2-64).

AL= light chain amyloidosis, AA= serum amyloid A amyloidosis, ATTR= wild type transthyretin amyloidosis.

b. AL amyloidosis patient characteristics and treatment details

In the 14 patients with systemic AL amyloidosis, 12 were renal transplant recipients (N=12/14, 86%), one a liver transplant recipient (N=1/14, 7%) and the final patient had a combined heart, lung and kidney transplant (N=1/14, 7%), see table 9.2. The reasons for transplantation are outlined in tables 9.1 and 9.2. The median age at diagnosis with AL amyloidosis was 50 (33-77 years) and the median time from transplantation to diagnosis with amyloidosis was 12 years (7 months- 31 years). A monoclonal paraprotein was detectable in 50% (7/14 cases) at a median value of 4.5g/l (range 3-28g/l). The

isotype was: IgG lambda in four cases, IgG kappa in two cases and IgA lambda in one case. The median concentrations of the amyloidogenic class of free light chain are outlined in table 9.2. Only one patient (N=1, 7%) was known to have had a monoclonal gammopathy of unknown significance prior to her transplant. Details of the underlying clone were available in 13/14 patients and this was a plasma cell clone in all cases.

Table 9.2: Light chain (AL) amyloidosis patient characteristics (N=14)

AL amyloidosis patients (N=14)	
Median age at diagnosis (years)	50 (33-77)
Median time from transplantation to diagnosis (years)	12 (0.58- 31 years)
Organ involvement	
Renal	10 (71%)
Spleen	8 (57%)
Cardiac	5 (36%)
Liver	5 (36%)
GI	0
Soft tissue	1 (7%)
Autonomic/peripheral nerve	0
Baseline	
Proteinuria g/24hrs	0.8 (0.3-5.9)
Creatinine µmol/L	251 (134-1124)
Albumin g/L	34 (27-49)
Bilirubin mmol/L	8 (2-22)
ALP U/L	95 (51-556)
cTNT ng/L	139.5 (<10-1000)
NT-proBNP ng/L	8060 (947-39951)
Presence of a monoclonal paraprotein (PP)	
Prior to diagnosis with amyloidosis	1
At diagnosis with amyloidosis	7
Median value of PP	4.5
PP isotype (N=7)	
IgG lambda	4
IgG kappa	2
IgA lambda	1
Baseline light chains (N %/ median (range)	
Kappa	4 / 1353.7 (406-1880)
Lambda	8 / 441.2 (32.2-1020)
Treatment (N=14, evaluable N =9)	
Bortezomib based	6
IMiD based	3
Melphalan	2
No treatment	1

Missing data	5
Haematological Response (N=8)	
CR	2
VGPR	1
PR	2
NR	2
PD	1
Renal response (N=6)	
Progression	2
Response	2
Not reaching either criteria	2
Not evaluable	0
Cardiac response (N= 5)	
Progression	2
Response	1
Not reaching either criteria	0
Not evaluable	2
Liver response (N=4)	
Progression	1
Response	0
Not reaching either criteria	2
Not evaluable	0
Median Overall survival (months)	23.5 (0-95)

GI= gastrointestinal, ALP= alkaline phosphatase, cTNT = high sensitivity cardiac troponin, NT-proBNP= N terminal B natriuretic peptide, PP= paraprotein, IMiD= immunomodulatory drug, CR= complete response, VGPR= very good partial response, PR= partial response, NR= no response, PD= progressive disease

Organ involvement with AL amyloidosis was as follows: kidney (N=10/14, 71.4%), spleen (N=8/14, 57.1%), heart (N=5/14, 35.7%), liver (N=5/14, 35.7%) and soft tissues (N=1/14, 7.1%). Treatment details were available for 9/14 patients, eight patients received chemotherapy aimed at their amyloidogenic clone, table 9.2. The median number of lines of treatment was one (range 1-2 lines). The most common chemotherapy was a bortezomib (N=6, 66.7%), followed by Thalidomide (N=2, 22.2%). Haematological response to treatment was assessed at 6 months; two patients (25%) achieved a complete response (CR) to treatment, one a very good partial response (VGPR) (12.5%), two a partial response (PR) (25%) two patients no response (NR) (25%) and one patient progressive

disease (PD) (12.5%). Organ responses were assessed at 12 months for the same eight patients and are outlined in table 9.2. Of the 15 patients with AL amyloidosis 11 (N=11, 79%) are either dead or have clinically relapsed with a median OS from diagnosis with amyloidosis of 23.5 months (0-95 months). In the eight patients who were treated, five patients (N=5, 63%) have progressed or died with a median progression free survival of 42 months (range 1-83 months).

c. AA amyloidosis patients

Of the 11 patients with AA amyloidosis two patients were recipients of a cardiac transplant (N=2/11, 18%) and the other 9 patients (N=9/11, 82%) were renal transplant recipients, see table 9.3. The median age at diagnosis was 57 years (40-73 years), and the time from transplant to the development of AA was 11 years (8 months-36 years). None of the patients had cardiac involvement, and the majority renal (N=7/11 63.6%) and splenic (N=6/11, 54.5%) involvement.

Table 9.3: AA amyloidosis patient characteristics (N=11)

AA amyloidosis patients (N=11)	
Age at diagnosis (years)	57 (range 40-73)
Time from transplantation to diagnosis (years)	11 (8 months-36 years)
Underlying inflammatory cause of AA:	
Bronchiectasis	2 (25%)
Recurrent infections	1 (12.5%)
Gout and hepatitis B	1 (12.5%)
Tuberculosis with an aspergilloma	1 (12.5%)
No clear cause identified	3 (38%)
Indeterminable (due to lack of clinical detail)	3 (27%)
Organ involvement with amyloidosis:	
Renal	7 (64%)
Spleen	6 (55%)
Cardiac	0
Liver	2 (18%)
GI	2 (18%)
Soft tissue	0
Autonomic/peripheral nerve	0
Unknown	1 (9%)
Baseline parameters	
Renal parameters:	
Proteinuria g/24hrs	1.5 (0-1-6.3)
Creatinine µmol/L	261 (71-692)
Albumin g/L	35 (26-50)
Liver parameters:	
Bilirubin mmol/L	7 (4-17)
ALP U/L	95 (32-225)
Cardiac biomarkers:	
cTNT ng/L	102 (69-205)
NT-proBNP ng/L	2803.5 (338-39418)
Inflammatory markers:	
SAA	43.9 (13-747)
CRP	22 (8-166)
Median Overall survival (months)	15 (0-77)

GI= gastrointestinal, ALP= alkaline phosphatase, cTNT = high sensitivity cardiac troponin, NT-proBNP= N terminal B natriuretic peptide, SAA= serum amyloid A; CRP= C reactive protein.

The median presenting serum amyloid P (SAA) and C-reactive protein (CRP) are outlined in table 9.3. The median time from diagnosis to death or last follow-up was 15 months (0-77 months). An underlying chronic inflammatory disorder was overt in 5/11 (45%) patients, see table 9.3; an underlying cause was not clearly identified in 3/8 cases (38%), and was indeterminable (due to lack of clinical detail) in 3 cases (27%).

d. wtATTR patients

The patient in this series with wtATTR amyloidosis was male and 67 years old at the time of diagnosis with a renal transplant four years earlier for diabetic nephropathy. His presentation was with breathlessness with an NT-proBNP of 12770ng/L and a cTNT of 172ng/L. The time from diagnosis with wtATTR to death in this patient was 17 months.

e. Localised AL patients

Three patients had localised AL amyloidosis. The first patient was 57 years when diagnosed with localised laryngeal disease after a renal transplant 11 years previous for polycystic kidney disease. The time from diagnosis to death was 64 months (5.3 years). The cause of death was unknown, but there was no evidence of systemic amyloid disease. The second patient was 36 years at diagnosis with localised lymph node amyloidosis following a liver transplant 5 years previously following a paracetamol overdose. The time from diagnosis to death was 20 months. The final patient was 43 years at the time of diagnosis with localised gastrointestinal amyloidosis following a renal transplant 10 years ago for end stage renal failure of an unknown cause. The patient's median graft survival was 189 months and the cause for graft failure was unknown, but amyloidosis was excluded. The patient declined further follow up at 67 months from diagnosis.

iii) Patient survival and renal transplant outcomes

The median follow-up was 21.5 months (range 0-95 months), defined from a diagnosis of amyloidosis to last follow up or death. In this time there were 19 deaths (63.3%). Detailed cause of death was not recorded. The median OS of all 30 patients, defined from date of diagnosis with amyloidosis to death or last follow-up was 45 months (range 2-88 months) and for each subtype of amyloidosis: localised AL, 64 months (20-67 months); systemic AL, 23.5 months (0-95 months); ATTR amyloidosis, 17 months; AA, 15 months (0-77 months).

Of the 25 patients with a kidney transplant, 11 patients had graft failure (44%). Median graft survival for all patients was 185 months (96-269 months) and in those patients whose grafts failed median graft survival was 2 months (range 2-64 months). The reasons for graft failure were available in 9/11 patients and are outlined in table 9.1.

9.4 Discussion

This series describes the characteristics of thirty patients who were diagnosed with amyloidosis following a solid organ transplant. This has not previously been described in the literature. Although there are clear grounds for concern about development of systemic AL amyloidosis post-transplant, as a complication of PTLD related production of monoclonal immunoglobulins, unexpectedly 11 patients

(37%) had AA amyloidosis, implying substantial chronic inflammation following transplantation and highlighting the importance of comprehensive investigation to establish the amyloid type.

One patient developed wtATTR (otherwise known as senile cardiac amyloidosis). In 2008, the estimated age adjusted incidence of wtATTR, based on new referrals to the NAC, was 0·3/100 000 population;(119) but this is likely an under-estimate reflecting substantial under-diagnosis.(173) Given the increasing number of patients recognised over the last decade, it is likely that a proportion of older patients with solid organ transplants will develop unrelated wtATTR as they age. Our patient with wtATTR had a presentation and disease course in keeping with wtATTR patients without a concurrent transplant, other than his slightly younger age at diagnosis (63 years, compared with the median age at presentation of 73 years)(174) and a worse survival than predicted by disease stage (17 months, compared with an expected 32.7 months (95% CI 23.4–37.0 months)).

Three patients (10%) were found to have localised AL amyloidosis post transplantation in the larynx, lymph nodes and gastrointestinal tract respectively. Localised disease usually has a good prognosis and does not require systemic chemotherapy again demonstrating the importance of a full amyloid work up prior to considering cytotoxic treatments. Localised amyloidosis is well recognised and assumed to be due to a focal clone of plasma cells within the local environment.

In a large case series 12% of 5050 new amyloid referrals were localised AL disease.(172) The median OS of these three patients was shorter than expected with a median OS of 2.8 years compared to a 10-year overall survival for all forms of localised amyloidosis 80.3% (75·7–84·1 months).(34) The patient with laryngeal involvement in this study had severe airway disease requiring a tracheostomy, radiotherapy and laser therapy to the airways which may explain the relatively short overall survival in this case. The other two cases are more complex; lymph node amyloidosis is almost always a complication of low grade lymphoma suggesting that this case was a form of PTLD. Localised gastrointestinal amyloid can also progress to systemic disease over time and, like lymph node amyloidosis, should be followed up carefully recognising the risk of progression.(34)

This series contains a surprisingly number of cases of AA amyloidosis. This was unexpected as AA amyloidosis is a rare condition, with an estimated incidence of one to two cases per million person-years.(13) Possible explanations for this finding include that AA amyloidosis was established but undiagnosed at the time of the solid organ transplant, i.e. was the unidentified cause of end stage renal failure. Against this, two of the 11 patients were recipients of a heart transplants (an organ rarely affected by AA type amyloid), and also the cause of end stage organ failure was established in 5/11 of the AA cases (eosinophilic granulomatosis with

polyangiitis, 2 hypertensive nephropathy, mesangiocapillary glomerulonephritis and reflux nephropathy). Of these diseases only, eosinophilic granulomatosis with polyangiitis is likely to be associated with significant ongoing systemic inflammation and a sustained hepatic acute phase response. This implies that AA amyloidosis developed following the solid organ transplant. In five of the 11 patients a chronic inflammatory condition was identified (bronchiectasis 2, gout with hepatitis B, recurrent infections and tuberculosis with an aspergilloma). In three patients the cause of chronic inflammation was occult, this is higher than the 28% that is quoted in the literature(13) and raises the possibility that long term transplant immunosuppression could predispose patients to either chronic infections or chronic inflammation of undetermined cause. Although transplant immunosuppression might be expected to at least partially control or ameliorate a number of commoner inflammatory conditions.

The development of AL amyloidosis in the post-transplant setting is less surprising as it a potential complication of PTLD. Systemic AL amyloidosis was found in 14 patients in this series with a further two patients with apparently localised amyloidosis in whom there is plausible concerns about indolent lymphoma. This could be an incidental finding, but AL amyloidosis, although about six fold more

common than AA amyloidosis, is still vanishingly rare with an incidence of five to twelve people per million person-years.(35)

The detection of a monoclonal gammopathy (MGUS) post-transplant is common, reported to be 10 fold higher than in the dialysis population. (175) Generally, the MGUS is thought to be transient and not increase the risk of developing a PCN or other plasma cell dyscrasias.(170, 176) The median age at diagnosis with AL amyloidosis in this study was only 50 years and yet the time from transplantation to a diagnosis of AL amyloidosis was 12 years suggesting that AL amyloidosis maybe a late complication perhaps reflecting an indolent form of PTLD. Without treatment progressive AL amyloidosis risks graft and eventually other organ failure and death. Outcomes were not favorable with an OS of 23.5 months and 79% of patients had died or clinically relapsed during the observation period. This presumably reflects the complexity of treating amyloidosis in the presence of a solid organ transplant; patients go into treatment relatively immunosuppressed and with a vulnerable graft which often has a limited functional reserve. Chemotherapy is of no benefit in other types of amyloidosis and the considerable treatment associated risks highlight the importance of definitive diagnosis of amyloid type and extent before embarking on treatment.

This study has a number of limitations; it is a retrospective analysis and, given the rarity of the condition, includes patients collected over a long period resulting in missing data. Details of the

immunosuppression taken at the time of diagnosis with amyloidosis were only available in 43% of cases, and any dose modification made to the immunosuppression regimen was lacking. This period was an era of dramatic change in chemotherapy regimens and precludes useful comparison between individuals or published outcomes of twenty-first century treatments.

In conclusion this case series is relatively large suggesting that transplantation is a genuine risk factor in the development of both AL and, strikingly, AA amyloidosis. In AL amyloidosis the most likely explanation is of a subtle PTLD(166) but the finding of 11 cases of AA amyloidosis raises questions about the extent of chronic inflammation in transplant recipients with potential severe consequences.

Chapter Ten

General Conclusions

The studies presented in this thesis have revealed novel findings in the prognosis, monitoring and management of AL amyloidosis and its complications.

The novel MALDI-TOF mass spectrometry (MS) technique outlined in chapter three confirmed that by using the unique molecular location of FLCs on MS, amyloidogenic FLC can be detected against a polyclonal, non-pathogenic FLC background. The technique will hopefully allow more accurate monitoring and more informed treatment decisions based on the monoclonal pathogenic FLC component. The ability of MS to analyse intact FLCs may also be crucial in capturing post-translational modifications, which may be key in the pathogenicity of FLC in AL amyloidosis and potentially also predict organ involvement.

The novel findings regarding the prognostication of patients with AL amyloidosis are outlined in chapter four. A small elevation in NT-proBNP (>152ng/L), as well as cardiac involvement by CMR, were identified as factors highly prognostic for survival in patients with AL

amyloidosis with Mayo stage I disease, and so no cardiac involvement by consensus criteria. This novel finding offers some insight into the heterogeneity in survival of Mayo Stage 1 patients with huge implications for clinical practice. In future, Mayo stage I patients may be categorised into: “low risk” and “high risk” stage 1 patients (with NT-proBNP < or >152 ng/L, respectively), with the goal of therapy for “high risk” patients being similar to those with cardiac AL i.e. a complete haematological response. The follow up of such “high risk” patients will then require serial NT-proBNP measurements and patients with NT-proBNP progression should be considered for further treatment.

Chapters five and six outline novel findings regarding the treatment of patients with AL amyloidosis. Chapter five confirmed the improved safety of ASCT with figures comparable to contemporary international data, supporting the continued use of ASCT for patients with AL amyloidosis. Chapter five also confirmed the validity of section criteria suggesting that Mayo stage, liver involvement and performance status are particularly important criteria for selecting eligible patients and that patients who achieve a deep haematological response to ASCT appear to benefit the most, with a prolonged clinical remission and excellent long term survival outcomes. Chapter six compared the outcomes of patients treated with an autologous stem cell transplantation versus bortezomib alone for patients with AL amyloidosis. This is a crucial and topical

question for clinicians which suggests that the benefit of ASCT may be minimal in the era of highly effective modern chemotherapy agents.

Chapter seven focuses on the treatment of relapsed real world AL amyloidosis patients treated with pomalidomide. The chapter outlines that a significant proportion of patients can achieve good haematologic responses, however responses are not as deep nor as durable in the real world setting. Responses are rapid and early responses appear to define longer term outcomes. The analysis also highlighted that pomalidomide is not as well tolerated in AL amyloidosis as myeloma and that careful dose titration may be required.

Chapter eight describes a CMV reactivation rate of 25%, corresponding to 39% of known seropositive patients with AL amyloidosis treated with bortezomib. No patients developed CMV disease but pre-emptive treatment was required in 36% of cases. This suggest bortezomib is associated with a substantial risk of CMV reactivation that warrants further evaluation.

The findings described in chapter nine highlight suggests that solid organ transplantation is a genuine risk factor in the development of both AL and, strikingly, AA amyloidosis. This is a previously

undescribed phenomenon. In AL amyloidosis the most likely explanation is of a subtle PTLD, but the finding of 11 cases of AA amyloidosis raises questions about the extent of chronic inflammation in transplant recipients with potential severe consequences. This important finding will hopefully make clinicians of amyloidosis as a possible diagnosis following solid organ transplantation which may facilitate a prompter diagnosis and treatment for patients with this complication of transplantation.

Chapter Eleven

Future studies

This thesis has identified a number of areas for future study, either to confirm, or expand on, the findings presented in this thesis hopefully in international collaborative or prospective studies.

The novel MS technique outlined in chapter three holds huge promise for the more accurate monitoring of AL amyloidosis patients FLCs over time. There are plans to extend the study described here to confirm the findings and to assess the impact of FLC-MS on survival and organ response outcomes.

The prognostic significance of NT-proBNP and cardiac involvement on CMR, in Mayo stage I patients, outlined in chapter four, have huge implications for clinical practice and a larger international collaborative study is already planned to confirm the findings and also to gather serial CMR data to delineate the natural history of such ‘high risk’ Mayo stage I patients and to help identify interventions to prevent progressive cardiac involvement.

The question of the ongoing benefit of ASCT in the setting of increasingly more efficacious and tolerable standard treatment agents is highly topical and controversial and the findings outlined in chapter seven require confirmation in a larger, and potentially international collaborative study.

The findings outlined in chapter eight, on the real-world outcomes of pomalidomide, have already been combined with an international collaborative study with the Italian amyloidosis group. Future studies should focus on the combination of pomalidomide with other agents like the anti-CD38 agent, daratumumab, as increasingly seen in the treatment of multiple myeloma, and the novel proteasome inhibitors, or venetoclax. All approaches may offer additional and deeper responses and needs future prospective study.

The findings of chapter nine have implications for how clinicians monitor patients on bortezomib treatment and future studies should focus on confirming the findings presented in this chapter, in a larger cohort of patients, including pre-treated/relapsed patients to delineate further the risk of CMV reactivation and CMV disease. This is particularly important in an era where increasingly complex combinations of immunomodulatory agents are being used to treat

increasingly more relapsed and immunocompromised myeloma/ amyloidosis patients.

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Appendix

Table 1: listed are patient variables which differed across the time cohorts. Logistic regression analysis was used to identify the time cohorts between which a variable differed with a corresponding p value/confidence interval for the difference.

Variable significantly different across cohorts	**Cohorts* that differ**	**P value for difference/ CI**
Age at time of ASCT (years)	1,3 1,4 2,3 2,4	<0.001 0.002 <0.001 0.001
Diagnosis to ASCT (months)	1,3 1,4	0.001 0.002
Performance Status (0/1 compared to 2/3/4)	1,3 1,4 2,3	<0.006 0.030 0.036
Number of organs involved (median)	1,3 1,4 2,3 2,4	0.023 <0.001 0.009 <0.001
Liver involvement	2,3	0.054
Baseline creatinine (µmol/l)	1,3 1,4	0.004 0.002
Albumin (g/l)	1,3	0.002
Proteinuria (g/dl)	3,4	0.007
Bilirubin (µmol/l)	1,4	0.002
ALP (IU/l)	1,3 1,4	0.019 0.026
ASCT line of treatment	1,2 1,3	0.009 0.001
Pre-ASCT chemotherapy regimen Thalidomide	2,3 3,4	0.96-> 3.90 -3.15->-1.21
Pre-ASCT chemotherapy regimen Velcade	2,3 3,4	-3.16->0.63
Conditioning Melphalan 200 Dose reduced melphalan	1,2	0.004
TRM (N (%))	1,4	0.004

*Cohort 1= 1994-2000; cohort 2=2001-2006; cohort 3= 2007-2012; cohort 4= 2013-2018. ASCT= autologous stem cell transplant; ALP= alkaline phosphatase; TRM= transplant related mortality.

Table 2: standardised differences of the variables used in matching to demonstrate the effect of propensity scoring matching between the bortezomib and ASCT groups

Variable N/median, (range/%)	**Bortezomib (n=68)** Mean/n (sd/%)	**ASCT (n=68)** n(%)/median(range)	**Standardised difference to test matching**
Performance Status			
0	29 (42.6)	26 (38.2)	0.10
1	34 (50.0)	36 (52.9)	
2	5 (7.4)	6 (8.8)	
Mayo Stage			
1	37 (54.4)	38 (55.9)	0.13
2	26 (38.2)	23 (33.8)	
3	5 (7.4)	7 (10.3)	
Organ Involvement			
Heart	0.26 (0.44)	0.279	-0.03
Number of organs involved(median)			0.30
1	39 (57.4)	43 (63.2)	
2	23 (33.8)	17 (25.0)	
3	4 (5.9)	7 (10.3)	
4	1 (1.5)	1 (1.5)	
5	1 (1.5)	0 (0)	
dFLC >180mg/l	0.397 (0.49)	0.43 (0.50)	-0.06
Age at treatment (years)	59.87 (9.13)	58.29 (6.58)	0.20
IVS (mm)	11.03 (2.05)	11.35 (1.98)	-0.16
NT-proBNP (ng/L)	2.56 (1203.7)	884.8 (1320)	-0.03
TnT	28.46 (28.54)	19.33 (27.05)	0.32
Bilirubin (µmol/l)	5.78 (3.87)	6.65 (2.94)	-0.25
ALP (IU/l)	118.2 (115.67)	88.82 (55.53)	0.32

IVS= left ventricular septal thickness; dFLC= difference in serum free light chains; NT-proBNP = N-terminal B natriuretic peptide; TnT= high sensitivity cardiac troponin; ALP= alkaline phosphatase.

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