A profile of AL amyloidosis with rare sub-types, novel investigations and prognostic markers

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I, Sajitha Sachchithanantham confirm that the work presented in this thesis is my own. I have declared where information has been derived from other sources.

Abstract

Background

Systemic AL amyloidosis is a rare complication of immunoglobulin light chain secreting B cell clonal disorders. Much progress has been made in the recent years in the management of AL amyloidosis. Yet, certain patient groups continue to fare badly, posing a challenge to the treating physicians.

Aims

To describe the clinical features and outcomes of the challenging subgroups of patients with AL amyloidosis such as elderly patients and those with rare subtypes – IgD and IgM related amyloidosis. To explore the role of ^{99m}Tc-DPD scintigraphy in imaging soft tissue AL amyloid deposits and look at possible risk stratifying methods based on plasma cell phenotype and serum clonal markers at presentation. To evaluate the effectiveness of the novel agent, bortezomib as front line therapy in AL amyloidosis.

Results and Conclusion

Treatment of systemic AL amyloidosis in the elderly is challenging, yet, treatment of carefully selected older patients with novel therapies with low toxicity profile, results in improved survival.

The clinical profile of IgD amyloidosis is similar to that of AL in general but the long term outcome appears poor. In contrast, IgM related amyloidosis has some distinct features and the underlying B cell clone needs to be accurately characterised to direct the choice of therapy. The adverse outcome in this latter group appears to be associated with cardiac, liver and nerve involvement.

The role of ^{99m}Tc-DPD scintigraphy in imaging soft tissue AL amyloidosis is promising and requires further studies. Multicolour flow cytometry and heavy light chain measurement seem valuable in assessing the impact of plasma cell clones and degree of immunosuppression on prognosis respectively.

Bortezomib based treatment is effective in achieving deep clonal response in patients without cardiac amyloidosis and those with early disease. Those with advanced cardiac involvement continue to pose a challenge and are in need of more effective therapies.

Ethical Approval

Explicit informed consent was obtained from all the individuals whose data has been used in the clinical research studies described in this thesis. Written consent was given by signing a consent form whilst visiting the National Amyloidosis centre. The consent form was approved by the Royal Free Hospital Ethics Committee (REC Ref 06/Q0501/42). The two international collaborative studies were performed with institutional review board approval, and informed consent was obtained from each patient in accordance with the Declaration of Helsinki. The dosage and administration of radioactive isotopes were approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health.

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Last but not least, the research in this thesis would not have been possible without the many patients and their referring physicians, for all of whom, I am especially thankful.

Contents

Abbreviations

β-2-microglobulin amyloidosis	β2M
Apolipoprotein A1	AApoA1
Apolipoprotein A-II	AApoAII
Cystatin C, variant	Acys
Fibrinogen amyloidosis	AFib
Gelsolin, variant	Agel
Immunoglobulin heavy chain	AH
Immunoglobulin light chain	AL
Leukocyte chemotactic factor 2	ALect 2
Alkaline Phosphatase	ALP
Lysozyme amyloidosis	ALys
Autonomic nervous system	ANS
Autologous stem cell transplantation	ASCT
Transthyretin amyloidosis	ATTR
Senile systemic amyloidosis	ATTRwt
Bone marrow	BM
Bone marrow plasma cells	BMPC

Bone marrow trephine biopsy	BMT
Cyclophosphamide, vincristine, doxorubicin and prednisolone	CHOP
Confidence interval	CI
Chronic kidney disease	CKD
Cladribine	CLAD
Cardiac magnetic resonance	CMR
Cyclophosphamide, vincristine and prednisolone	COP
Complete response	CR
Cyclophosphamide, lenalidomide and dexamethasone	CRD
Computerised tomography	CT
Cyclophosphamide, thalidomide, dexamethasone	CTD
Cyclophosphamide, thalidomide and attenuated dexamethasone	CTDa
Cardiac troponin-I	cTnI
Cardiac troponin-T	cTnT
Cyclophosphamide, bortezomib and Dexamethasone	CyBorD
Difference between the amyloidogenic and uninvolved SFLC concentration	dFLC
Deoxyribonucleic acid	DNA
Electrocardiogram	ECG

Eastern Cooperative Oncology Group	ECOG
Ethylenediaminetetraacetic acid	EDTA
Estimated glomerular filtration rate	eGFR
Equilibrium contrast CMR	EQ-CMR
End stage renal disease	ESRD
Familial Amyloid Polyneuropathies	FAP
Fludarabine and cyclophosphamide	FC
Fludarabine, cyclophosphamide and Rituximab	FCR
Fluorodeoxyglucose	^{18}F-FDG
Fluorescence in situ hybridisation	FISH
Gastrointestinal system	GI
Heavy and light chain	HLC
Hematologic response	HR
High resolution CT	HRCT
International consensus criteria	ICC
Intermediate dose melphalan	IDM
Immunofixation electrophoresis	IF / IFE
Immunoglobulin-A	IgA
Immunoglobulin-D	IgD

Immunoglobulin-G	IgG
Immunoglobulin-M	IgM
Immunomodulatory agent	IMiD
Interquartile range	IQR
Intent to treat analysis	ITT
Interventricular septum	IVS
Kappa	κ
Lambda	λ
Lenalidomide and dexamethasone	LenDex
Liver function test	LFT
Laser microdissection	LMD
Lymph node	LN
Lymphoplasmacytic lymphoma	LPL
Left ventricle	LV
Left ventricular hypertrophy	LVH
Monoclonal antibodies	mAb
Melphalan and dexamethasone	MDex
Multicolour flow cytometry	MFC
Monoclonal gammopathy of undetermined significance	MGUS

Monoclonal intact immunoglobulins	M-Igs
Multiple myeloma	MM
Melphalan and prednisone	MP
Melphalan, prednisolone and cyclophosphamide	MPC
Melphalan, lenalidomide and dexamathasone	MRD
Minimal residual disease	MRD
Magnetic resonance imaging	MRI
Mass spectrometry	MS
National Amyloidosis Centre	NAC
Non-Hodgkin’s lymphoma	NHL
Not reached	NR
N-terminal pro-natriuretic peptide type B	NT-proBNP
New York heart association functional classification	NYHA
Overall survival	OS
Plasma cell dyscrasia	PCD
Polymerase chain reaction	PCR
Positron emission tomography	PET
Peripheral neuropathy	PN
Peripheral nervous system	PNS

Partial response	PR
Rituximab and Bortezomib	RBortezomib
Rituximab, cyclophosphamide and dexamethasone	RCD
Rituximab and chlorambucil	RCHL
Rituximab, cyclophosphamide, vincristine, doxorubicin and prednisolone	RCHOP
Rituximab, cyclophosphamide, vincristine and prednisolone	RCVP
Rituximab and Purine Analogues	RPA
Serum amyloid A protein	SAA
Serious adverse events	SAE
Serum amyloid P component	SAP
Systolic blood pressure	SBP
Stem cell transplantation	SCT
Serum electrophoresis	SEP
Serum free light chain assay	SFLC
Single photon emission computed tomography	SPECT
^{99m}Technetium-3,3,-diphosphono-1,2-propanodicarboxylic -acid scintigraphy	^{99m}Tc-DPD
^{99m}Technetium-methylene diphosphonate	^{99m}Tc-MDP

^{99m}Technetium-labeled pyrophosphate	^{99m}Tc-PYP
Thalidomide and dexamethasone	ThalDex
Treatment related mortality	TRM
Time to next treatment	TTNT
Vincristine, Adriamycin and dexamethasone	VAD
Very good partial response	VGPR

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Chapter One: Introduction

This chapter is written in the context of two of my publications:

Imaging in systemic amyloidosis. Sachchithanantham, S., & Wechalekar, A. D. (2013). Review article. Br Med Bull. 2013;107:41-56. Copyright permission obtained from Oxford University Press, licence no. 4036760241088 for use in my thesis

An evaluation of current treatment options for AL Amyloidosis - Review article. Sachchithanantham, S., & Wechalekar, A. D. and Hawkins, P. N. Expert opinion in orphan drugs. 2014:2 (3) pp. 229-244. Copyright permission obtained from publisher for use in my thesis.

What is Amyloidosis?

Amyloidoses are a heterogeneous group of diseases resulting from unstable circulating proteins which give rise to extracellular deposition of insoluble amyloid fibrils. The condition leads to gradual tissue destruction and eventual organ dysfunction. Amyloid deposition can occur in any organ: heart, lungs, liver, kidneys, skin, bones, peripheral or autonomic nerves are commonly involved. There are currently over 30 different proteins known to cause amyloidosis (Table 1.1).[1] Amyloidosis can be classified in several ways; hereditary or acquired, by means of the precursor proteins and the distribution

of amyloid deposits (localised or systemic). The natural history and outcome are dependent on the amyloid fibril type and anatomical distribution.

Amyloid fibril and fibrilogensis

The molecular structure of amyloid fibril and the mechanism underlying its formation are not completely understood. Despite having heterogeneous structures and functions, all amyloid proteins can generate morphologically indistinguishable amyloid fibrils.[2] In the light-microscope amorphous and homogenous amyloid, irrespective of type, consists of fine, 10nm thick fibrils.[3] Other constant components include proteoglycans (especially heparin sulphate), the glycoprotein serum amyloid P component (SAP), apolipoprotein E, laminin and Collagen IV.[4] The protein monomers in amyloid are bound to each other by hydrogen bonds to a very stable intermolecular β-sheet. It is this characteristic β pleated sheet configuration that produces apple green birefringence under polarised light when stained with Congo red dye. The protein monomers are orientated perpendicularly to the fibril axis forming a thin filament. Several filaments then twist around each other to form a definite amyloid fibril. This structure is shared by all types of amyloid despite the high diversity between amyloid fibril proteins.[5]

In vivo there are a limited number of proteins which are able to form amyloid fibrils. One crucial component is the β-structure, either present in normal folded state of the amyloidogenic protein or acquired during amyloidogenesis. Examples of proteins with β-structure in their normal folded state include β-2 microglobin, immunoglobulin light chains and transthyretin.[6-8]

Other proteins such as apolipoproteins, including serum amyloid A protein (SAA) have comparatively little β-structure in their normal folded state and undergo α-helix to β sheet conversation.[9] A number of factors affect the conformational transition from a native protein into pathological aggregates. The main factors include: high concentration of normal proteins such as that seen with SAA in chronic inflammatory conditions predisposing to AA amyloidosis; the protein's intrinsic propensity to assume pathologic conformation that become evident with aging as seen with transthyretin in senile systemic amyloidosis (ATTRwt); and mutations as occurs in hereditary amyloidosis where the substitution of a single amino acid transforms a normal protein into an amyloidogenic one such as in transthyretin (ATTR), fibrinogen α chain (AFib), apolipoprotein A1 (AApoAI) and lysozyme amyloidosis (ALys).[10] Physiologic mutations that occur during immune response in the variable domains of immunoglobulin light chains can sometimes affect critical structural sites and destabilise the domain favouring the generation of an aggregation prone state as seen in AL amyloidosis.[11, 12]

Amyloid deposition and degradation

The distribution of amyloid deposits in organs varies significantly between amyloid fibrils. Fibrinogen α chain predominantly aggregate in the kidneys, transthyretin Met30 variant in peripheral nerves and β2-microglobulin in joints and light chain amyloidosis can involve any organ but the brain. In hereditary amyloidosis, there may be further phenotypic variation within the same families with a specific genetic mutation. The site of deposition may depend on the

coexistence of several factors favouring the formation of fibrils, such as local protein concentration, a low pH and other factors.[13] For example, in AL amyloidosis, recognition of particular tissue constituents (i.e., collagen) by amyloidogenic light chains may determine the specificity of tissue deposition.

The mechanism by which amyloid formation results in tissue damage and organ dysfunction is not well understood and is believed to be beyond mechanical replacement of parenchymal tissue by large amounts of amyloid deposits. Some studies suggest that in ATTR amyloidosis, it is the prefibrillar oligomers, rather than the fibrillar form, as the major pathologic species.[14, 15] Others have demonstrated direct cytotoxicity of amyloidogenic immunoglobulin light chains in AL amyloidosis, especially in cardiac cells.[16] This hypothesis is supported by improvements in cardiac function after arresting the production of amyloidogenic light chains with chemotherapy but before any evidence of improvement in myocardial amyloid deposits on echocardiogram.[17] The degree of cytotoxicity and tissue dysfunction caused by the amyloid deposits or prefibrillar aggregates may vary between types of amyloidosis and involved organs.

Amyloid formation, deposition and tissue damage occurs in the presence of high levels of circulating amyloidogenic proteins. Organ dysfunction can be halted or reversed if the production of the precursor protein is suppressed.[18, 19] This is based on the principle that oligomers form at a certain threshold and that reducing the concentration of the amyloidogenic protein without the need to eliminate it, will promote the resorption of amyloid deposits. The exact mechanism of amyloid resorption is still unclear. It has been postulated that macrophages may have a role in amyloid regression. Studies of Amyloid-β

immunotherapy suggest phagocytosis may have a role but that only when additional production of Aβ is arrested can it make an impact on amyloid burden.[20] The heterogeneity in the rate of regression of amyloid amongst patients despite complete suppression of amyloidogenic precursor protein may be explained by variation in phenotype and function of macrophages and monocytes.

Table 1.1 Classification of Amyloidosis by implicated precursor proteins and distribution

Amyloid type / Fibril protein	**Precursor**	**Acquired or Hereditary**	**Systemic / Localised**	**Clinical Syndrome**
Immunoglobulin light chain (AL)	Monoclonal immunoglobulin light chains	A	S, L	Associated with monoclonal plasma cell dyscrasias
Immunoglobulin heavy chain (AH)	Monoclonal immunoglobulin heavy chains	A	S, L	Associated with monoclonal plasma cell dyscrasias
Reactive amyloidosis (AA)	Serum amyloid A	A	S	Associated with chronic inflammation, infection, or certain neoplasia
B2-microglobulin (Aβ2M)	B2-microglobulin, wild type	A	L	Associated with chronic haemodialysis (affects Musculoskeletal system)
Senile systemic amyloidosis (ATTRwt)	Transthyretin, wild type	A	S	Age-related, usually males (primarily cardiac involvement)
Transthyretin amyloidosis (ATTR)	Transthyretin, Variant, > 100 amyloidogenic mutations	H	S	PNS, ANS, Heart, eye, leptomen
Fibrinogen amyloidosis (AFib)	Fibrinogen α chain, variant	H	S	Primarily kidneys
Apolipoprotein A-I (AApoAI) Amyloidosis	Apolipoprotein A-I, variants	H	S	Heart, liver, kidneys, skin, larynx, testes
Apolipoprotein A-II (AApoAII)	Apolipoprotein A-II, variants	H	S	Kidneys
ALys	Lysozyme, variant	H	S	Kidney, liver, spleen
AGel	Gelsolin, variant	H	S	Cranial nerve involvement with lattice corneal dystrophy
ACys	Cystatin C, variant	H	S	Icelandic hereditary cerebral amyloid angiopathy
ALect 2	Leukocyte chemotactic factor 2	H	S	Slowly progressive with kidney and liver involvement

Types of Amyloidosis

Systemic amyloidosis

The systemic amyloidoses affect various organ systems and are caused by a number of precursor proteins as listed on Table 1.2.

Table 1.2 Typical organ involvement in various types of systemic amyloidosis.[21]

Type of amyloidosis	Cardiac	Renal	Liver/GI tract	PNS	Soft tissue
AL	✓	✓	✓	✓	✓
Hereditary ATTR	✓	Uncommon	✓	✓	
ATTRwt	✓				
AA	Uncommon	✓	✓		
AFib	Uncommon	✓	✓		
AApoA1	✓	✓	✓	✓	
ALys		✓	✓		Uncommon
AGel		Uncommon		✓	✓

The International Society of Amyloidosis has devised a nomenclature based on the nature of the main protein that constitutes the amyloid fibril. The fibril proteins involved in the main forms of systemic amyloidosis are designed as follows: AL is the protein derived from immunoglobulin light (L) chains; AA the fibril protein derived from the acute phase protein SAA; and ATTR the fibril protein derived from the plasma protein transthyretin. Systemic light chain (AL) amyloidosis is the most common of these conditions, but ATTRwt cardiac amyloidosis is increasingly being diagnosed.

AL Amyloidosis

Immunoglobulin light chain (AL) amyloidosis is the most common of the systemic amyloidosis. It is characterised by the deposition of amyloid fibrils derived from the aggregation of misfolded, kappa or lambda monoclonal immunoglobulin light chains.[22] The abnormal folding results from either a proteolytic event or an amino acid sequence which renders a light chain thermodynamically unstable and prone to self-aggregation. The aggregates form protofilaments that associate into amyloid fibrils.[23] The light chains are produced by a B-cell clone, which in the majority of cases is a plasma cell clone. In a few patients, however, the amyloid deposits have been reported to contain immunoglobulin heavy chains and are therefore termed H chain type amyloidosis (AH). It is the toxic products of the B-cell clone rather than its malignant behaviour that are thought to be responsible for the fatal consequences of AL amyloidosis. The actual mechanism whereby amyloid deposits produce organ dysfunction remains unclear but theories include two main mechanisms, firstly via disruption of tissue architecture from the accumulation of amyloid fibrils and secondly through direct toxicity of the pre-fibril oligomers.[24]

The incidence of AL amyloidosis is around five to twelve people per million person-years, although autopsy studies suggest that the actual occurrence might be higher.[25] The median age at diagnosis is 63 years, and median survival if left untreated is 12 months.[26]

AL amyloidosis can affect any organs apart from the central nervous system. Patients usually present with a wide range of symptoms that are often mimicked by more common disorders, which, inevitably leads to delayed

diagnosis in the absence of high grade of suspicion. Kidneys and the heart are the most commonly affected organs.[27, 28] Amyloid deposits in the heart result in restrictive cardiomyopathy. Heart failure is a rapidly progressive complication.[28] Symptoms are those of congestive cardiac failure, most commonly breathlessness.[29] Renal involvement usually presents with nephrotic syndrome and worsening renal function. Severe cases of amyloid infiltration of the liver sinusoids result in liver failure. AL amyloidosis also affects both the autonomic and peripheral nervous system. Involvement of the former results in orthostatic hypotension and symptoms due to delayed gastric emptying and impaired intestinal motility. Patients also suffer from erectile dysfunction. Peripheral nervous system involvement causes bilateral, distal, symmetrical, painful sensory symptoms before progressing to motor neuropathy. Orthostatic hypotension can also be secondary to intravascular depletion in nephrotic syndrome and is a common feature in cardiac amyloidosis. Soft tissue infiltration may manifest in a number of ways, including macroglossia, carpal tunnel syndrome, skin nodules, arthropathy, alopecia, nail dystrophy, submandibular gland enlargement, periorbital purpura and hoarseness of voice. Macroglossia and periorbital bruising are hallmarks features of AL amyloidosis, although rare. Amyloid infiltration of the thyroid and adrenal glands are rare and result in hypothyroidism and hypoadrenalism respectively.[27] Patients can also develop acquired factor X deficiency[30, 31] which can be a hindrance to performing biopsies necessary to confirm the diagnosis. The exact mechanism of factor X deficiency is unclear but include adsorption on amyloid fibrils, synthetic dysfunction due to liver involvement and vitamin K deficiency.[32, 33] Although baseline factor X levels are not predictive of bleeding risks[34], factor X

assays are important in patients with abnormal clotting at baseline and for monitoring response to replacement and treatment of AL amyloidosis.

The survival of AL amyloidosis has improved over time, with maximum improvement over the last decade attributable to better supportive care and novel chemotherapeutic agents. Mayo clinic reported an improvement in the four year overall survival (OS) from diagnosis during each decade between 1977 and 2006. The four year survival between 2003-2006 was 42% compared to 30% between 2000-2002 and 21% between 1977-1986. Interestingly, the one year mortality during this 30 year period remained high.[35]

IgM-related AL amyloidosis

The majority of patients with AL amyloidosis have an underlying plasma cell dyscrasia (PCD), but in 5-6% of patients, the underlying clone is of lymphoplasmacytic (LPL) origin producing an IgM paraprotein. The level of bone marrow infiltrate can be very subtle in these patients, making the detection of the underlying clone challenging at times. Traditionally patients with IgM-related AL amyloidosis have been treated similarly to those with underlying PCD, but in recent years, treatment has been tailored to the differing nature of the underlying B cell disorder. Currently, patients with IgM-related AL amyloidosis on a background of an underlying LPL, are treated with a rituximab based therapies similar to those use in low grade non-Hodgkin's lymphomas.

AA Amyloidosis

The amyloid fibrils of AA amyloidosis are composed mainly of the serum amyloid A (SAA) protein. SAA is usually present at low levels in serum and is synthesised by hepatocytes in response to various pro-inflammatory cytokines.[36] Persistently elevated levels of SAA is essential for the development of AA amyloidosis but only a small number of patients with inflammatory conditions eventually develop amyloidosis.[37] The estimated global incidence of AA amyloidosis in the UK is one case per million person-years.[38]

AA amyloidosis most commonly affects the kidneys. The earliest clinical manifestation is proteinuria, which eventually leads to nephrotic syndrome and renal insufficiency. Proteinuria may be present in up to 95% of patients and determines prognosis.[39] Other commonly affected organs are liver, spleen and gastrointestinal tract, usually without any clinical significance in the early stages. Splenic involvement is demonstrable on SAP scintigraphy in almost all patients with AA amyloidosis. AA amyloidosis is rarely known to affect the heart, cause soft tissue infiltration or peripheral neuropathy.

Common therapeutic approaches are limited in AA amyloidosis due to the diversity of the underlying conditions that can cause the disease. Complete suppression of inflammation with SAA concentration persistently <5mg/L, can lead to regression of amyloid deposits and preservation of renal function.

The use of intensive treatment protocols and the availability of biologics have essentially modified the natural history of inflammatory joint disease in developed countries.

Wild type transthyretin amyloidosis or Senile Systemic Amyloidosis

Wild-type transthyretin amyloidosis invariably affects older patients. Hence, it is also known as senile systemic amyloidosis and is a slowly progressive disease. The amyloid fibril is composed of normal wild-type transthyretin.[40] The true incidence of clinically significant myocardial deposits of ATTRwt remains unknown. Its prevalence increases with age and has a male preponderance. The burden of disease is likely to become an increasing problem as the population demographic shifts toward the elderly in the current era of sophisticated cardiac imaging techniques such as cardiac magnetic resonance (CMR).[41] The heart is the most predominantly involved organ but deposits are also seen in other sites with limited clinical significance.[42] Heart failure is the most predominant presenting feature.[43] Patients with ATTRwt are reported to have greater left ventricular (LV) wall thickness than those with cardiac AL and hereditary TTR amyloidosis.[44] ^{99m}-technetium-3,3,-diphosphono-1,2-propanodicarboxylic acid (^{99m}Tc-DPD) scintigraphy has been shown to be a sensitive imaging technique in diagnosing cardiac ATTR amyloidosis.[45]

The principle of current management is mainly supportive with emphasis on heart failure symptoms involving meticulous fluid balance control, aided by heart failure team and management of arrhythmias.

The Hereditary Systemic Amyloidoses

Hereditary amyloidoses are rare and result from mutation in genes encoding variant proteins giving rise to new proteins with amyloidogenic properties. All types of hereditary amyloidosis are dominantly inherited but many patients have

no obvious family history. Age of onset, disease penetrance and phenotype vary between different mutations and even within families, posing a challenge for genetic counselling.

Hereditary amyloidoses can be divided into neuropathic and non-neuropathic forms. Clinically, the syndrome maybe indistinguishable from AL amyloidosis. The non-neuropathic forms include AFib amyloidosis, AApoAI, AApoAII, and ALys amyloidosis. This group of diseases typically present with renal dysfunction in association with involvement of various other organ systems.[46-48] Other hereditary forms of amyloidosis include cystatin C (ACys) and β-2-microglobulin (β2M) amyloidosis. The neuropathic hereditary systemic amyloidoses comprise the Familial Amyloid Polyneuropathies (FAP), commonly caused by mutations in the transthyretin gene (ATTR) and rarely AGel amyloidosis. Patients with hereditary ATTR amyloidosis present with neuropathy and cardiomyopathy, although renal amyloid deposits occasionally associated with end stage renal disease (ESRD), maybe present.[49] Patients with AGel amyloidosis present with cranial neuropathy.

The mainstay of diagnosing the hereditary amyloidosis is DNA testing. The choice of the gene to sequence is guided by the clinical presentation and the results of histology and immunohistochemistry where available.[50] The diagnosis of a hereditary form of amyloidosis with its implications for future generations can be devastating news to the patient. Therefore, genetic counselling is of great importance in hereditary amyloidosis.

Localised AL amyloidosis

In localised amyloidosis, the amyloid deposition is confined to a specific organ or site. The commonest reported localised amyloidosis is AL type and is characterised by localised growth of monoclonal plasma cells. This is not part of a systemic AL amyloidosis where the amyloid light chains are produced by bone marrow. Infiltration of plasma cells has been observed near localised amyloid deposits suggesting local production of the amyloid fibril precursor protein.[51] It can appear almost anywhere.[52-56] The commonly reported sites include the urinary tract (bladder, urethra and ureter) and the respiratory tract (larynx and tracheobronchial tree).[57, 58] Unlike in systemic AL amyloidosis, the clinical consequence of localised type is determined by the site and the extent to which the amyloid fibrils are deposited – typically causing problems due to mechanical obstruction. Localised amyloidosis rarely progresses to become systemic. It has an excellent prognosis with no apparent effect on life expectancy.[52] The condition, may, however, cause substantial morbidity and affect quality of life due to complications such as haemorrhage and dyspnoea.

The management of localised amyloidosis is determined by the site involved and the symptoms experienced by the patient. Treatment is mainly localised and in most cases experimental due to the paucity of localised amyloidosis and diversity of affected sites. It is very rarely that patients with localised amyloidosis require systemic chemotherapy.[52]

Although, localised amyloidosis rarely evolve to a systemic form, patients should undergo long term follow-up in order to monitor the localised disease

and for the early detection of any systemic manifestations leading to prompt treatment.[52, 58]

Diagnosis of AL amyloidosis

Histology - Typing Amyloid deposits

Correctly identifying the amyloid type is vital, as it has a major impact on prognosis and dictates treatment. The amyloid fibril type is difficult to deduce clinically in any given patient as the clinical presentations are similar for the various types of amyloidosis. Biopsy followed by Congo red staining under polarised light and immunohistochemistry is the gold standard for diagnosing and typing amyloid.[59] Majority of patients have one or more organ involvement, and biopsies of clinically involved organs such as kidneys, endomyocardium and liver are invasive and have high risk of post procedure haemorrhage.[60] The bleeding risk after liver biopsy is 2%.[61] Biopsy proof of amyloid at an alternate site such as subcutaneous fat, bone marrow, rectum, labia minor, or salivary gland biopsy carry a lower risk and can be helpful in diagnosing amyloid. Amyloid deposits can be identified in 85% of patients with a combination of bone marrow and abdominal subcutaneous fat aspiration.[62] Biopsy of the suspected affected organ may become necessary when both the bone marrow and fat biopsy fail to identify amyloid deposits.

Fibril type is most commonly confirmed with immunohistochemistry. High background staining makes this unreliable in a third of patients with AL

amyloidosis.[63] Another problem is two potential precursor proteins coexisting in a patient, making it clinically perplexing to elicit the type of amyloidosis present. This is particularly relevant in the African-American population, who have higher rates of monoclonal gammopathies and 4% carry a variant of transthyretin (Val122Ile), and in elderly men, who also have higher rates of both monoclonal gammopathies and can develop wild-type ATTR amyloidosis in the heart.[50, 64] In these cases, patients almost always have one type of amyloid causing the disease, and confluent risks exist for both doctor and patient. A tissue biopsy, often endomyocardial, is critical in this situation. It is also important to exclude secondary or hereditary amyloidosis as PCD maybe an incidental finding. Recent advances in diagnostic testing such as mass spectrometry can help identify the type of amyloidosis with high reliability and accuracy. Amyloid fibril typing can also be performed on immune-electron microscopy which is highly specific but has limited availability.[65]

Mass spectrometry

Laser microdissection of the Congophilic deposits followed by mass spectrometry (LMD/MS) with customized bioinformatics assessment of the constituents may enable precise identification of type in over 98% of cases.[66] LMD/MS is specifically indicated for typing in any case where the immunohistochemistry is unrevealing and may be particularly useful in cases in which two potential amyloid precursor proteins are present in a patient. A critical feature of LMD/MS is that it captures all of the chaperone and fellow-

traveller elements in amyloid deposits, as well as the identity of the protein in the fibrils.

DNA analysis

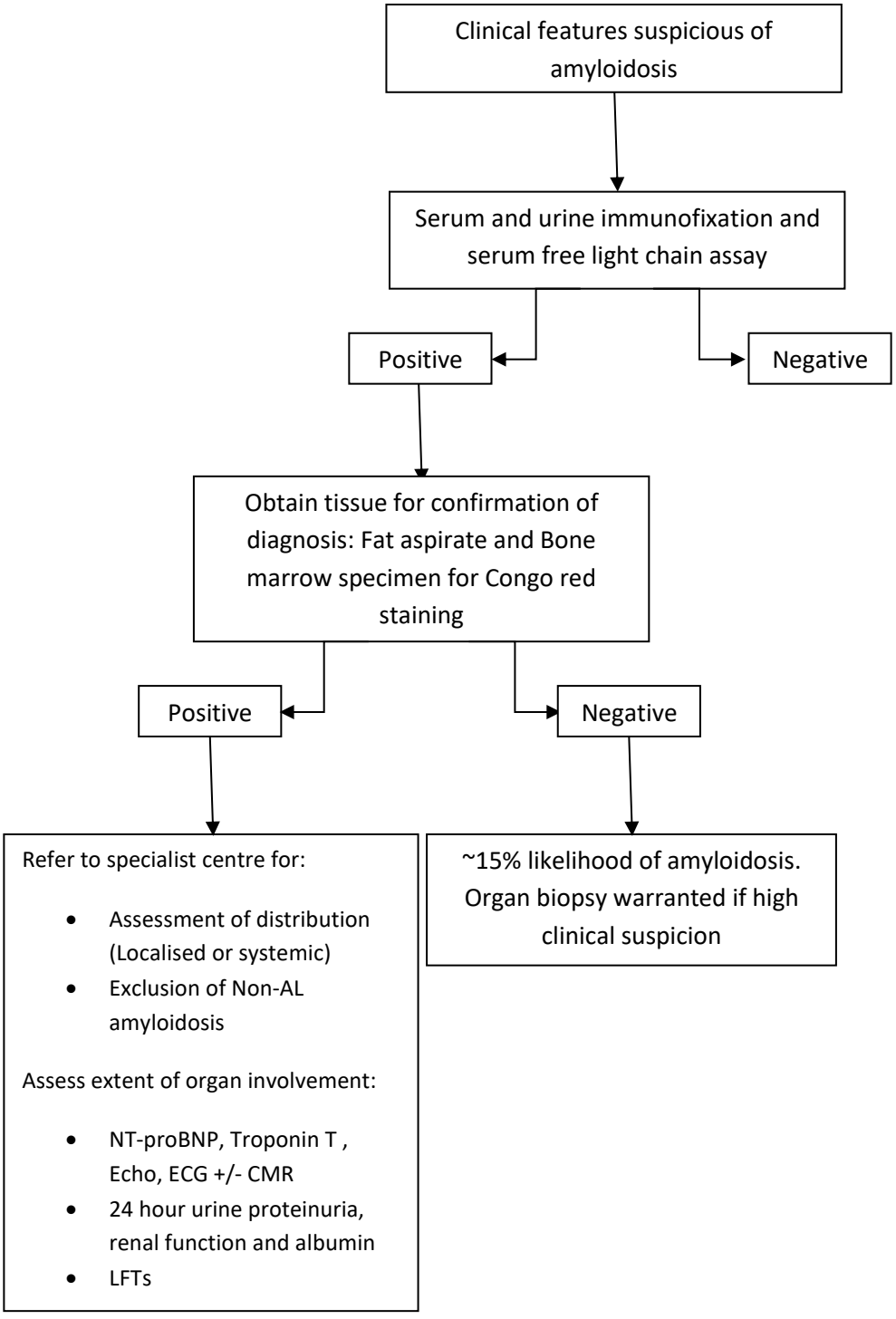
The clinical features in hereditary systemic amyloidosis may be indistinguishable from those in AL amyloidosis. DNA analysis helps differentiate between the two. Hereditary amyloidoses are autosomal dominant conditions but a family history of amyloidosis may be absent due to incomplete penetrance. Consequently, occasionally, new variants and new amyloidogenic proteins are identified. There should be a low threshold for sequencing the TTR gene in patients with polyneuropathy and/or amyloid cardiomyopathy as hereditary ATTR amyloidosis presents with similar phenotype. Likewise, hereditary AFib amyloidosis should be considered in any patients with isolated renal involvement. Once the gene defect is identified, screening and counselling must be offered to relatives.[67] Patients with AL amyloidosis may occasionally have an incidental mutation therefore genetic results must be interpreted in the context of clinical and histological findings.[50, 68] DNA analysis is also essential in patients with systemic amyloidosis whose fibril type cannot be confirmed by immunohistochemistry or mass spectrometry.

Assessment of Organ Function

Amyloidosis can be either localised or systemic; therefore, on confirming the presence of amyloid deposits, it is imperative to investigate the distribution of

the amyloid deposits and severity of organ involvement. Assessment should also include prognostic stratification as this guides treatment. Figure 1.1 shows an algorithm for evaluating patients with suspected amyloidosis.

Figure 1.1 Algorithm of investigation of patients with clinical suspicion of amyloidosis.[21]



Biochemical analysis

The first international consensus opinion for the definition of organ involvement and response to treatment was published in 2005.[69] These criteria were recently updated and form the basis for data collection and reporting, including clinical trials.[70] In addition to biopsy confirmation of the suspected organs where necessary, clinical and biochemical evidence of organ dysfunction form the mainstay of definition of organ involvement.

The extent of cardiac involvement is the major determinant of outcome in AL amyloidosis. Approximately 60% of patients with AL amyloidosis present with heart involvement, and about 80% die a cardiac death.[71] The degree of cardiac damage determines survival and treatment tolerability. The definition of cardiac involvement has been better defined with the introduction and wide availability of cardiac biomarkers: serum troponin T and N-terminal pro natriuretic peptide (NT-proBNP). Troponin I or T provides a quantitative assessment of cardiac damage and BNP and/or NT-proBNP indicate cardiomyocyte stress and higher levels are independently associated with poorer survival.[72] The Mayo staging system[73] using NT-Pro BNP (0.332ng/L) and cardiac troponin-T (cTnT)/troponin-I (cTnT 0.035mcg/mL; cardiac troponin-I, 0.1 ng/mL) is the most robust and widely used method for risk stratification. Patients are categorised into three stages. Patients with stage III disease have the poorest prognosis with a median survival of 3.5 to 8 months.[73-75] This staging system is important for clinical management, but also for stratifying patients enrolled on clinical trials. As these markers are elevated in chronic kidney disease and other cardiac conditions, they should be interpreted with

caution. In patients with renal dysfunction with eGFR <30ml/min, Mayo staging is not directly applicable.

Renal involvement and the degree of dysfunction are best evaluated by eGFR and albuminuria. Disease progression can be monitored with quantification of 24-hour urine protein loss. The urine protein is predominantly albumin, unlike in multiple myeloma (MM) where large amount of immunoglobulin light chains are excreted. Other causes of albuminuria should be excluded and approximately 10% of patients present with renal dysfunction and non-nephrotic range proteinuria.[76]

In addition to non-tender hepatomegaly, liver function tests are useful to document involvement. Damage due to hepatic amyloid is usually obstructive in nature and should be suspected in patients when serum alkaline phosphatase value is 1.5 times the upper limit of the institutional normal value. However, significant amyloid infiltration of the liver would have occurred by this time. An obstructive liver dysfunction and hepatomegaly may also be due to right heart failure from cardiac amyloidosis.

Imaging

SAP Scintigraphy

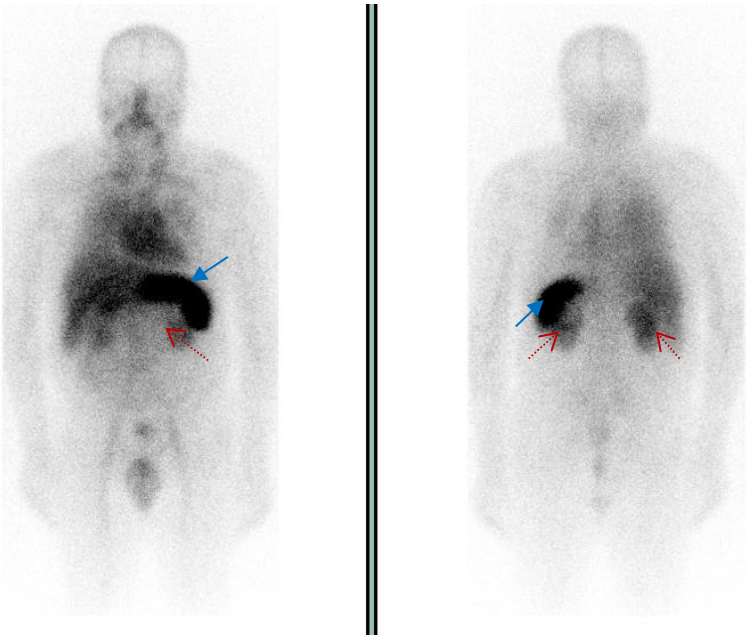
SAP is a non-fibrillar glycoprotein of the pentraxin family and binds amyloid independently of the protein of origin. It has a specific binding motif for the common conformation of amyloid fibrils. The binding of SAP is reversible and calcium dependent. This property makes radiolabelled SAP a diagnostic tool for the imaging of amyloid deposits.[77] SAP in circulating plasma is in constant

equilibrium with SAP in the amyloid deposits. Pepys and Hawkins developed the theory of using radiolabelled (^{123}I) SAP as a tracer imaging for amyloid deposits.[77, 78] When injected radiolabelled SAP localises rapidly and specifically to amyloid deposits in proportion to the quantity of amyloid deposited. The dose of radiation is small and comparable to a plain X-ray of the lumbar spine.[78] A number of studies over the years have confirmed that this is a safe and non-invasive technique providing information on the presence, distribution and extent of amyloid deposits of all types and its utility in monitoring of treatment responses.[79] SAP scintigraphy is reported to have 90% sensitivity in AA and AL amyloidosis.[80]

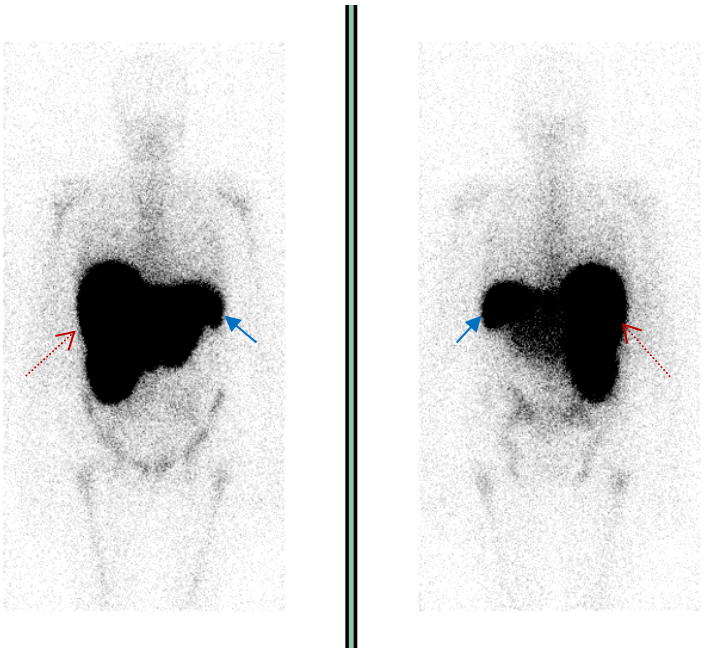
SAP scintigraphy enables identification of amyloid deposits[78] in the liver, kidneys, spleen, adrenal glands and bones. It is valuable in identifying amyloid deposits in organs that have not been suspected clinically (e.g. liver or adrenals) and in anatomic sites that are not available for biopsy (e.g. spleen). SAP scintigraphy will identify liver involvement in over 30% of cases not detected by standard tests of liver function or size (Figure 1.2).[81] The patterns of organ involvement on SAP scintigraphy may give clues to, but is not diagnostic of, the amyloid fibril type since there is considerable overlap in the patterns of organ involvement. Significant uptake in the bones is a feature almost unique to AL amyloidosis.[78]

Figure 1.2 Whole body ^{123}I-SAP scintigraphy; **a)** Anterior and posterior whole body scan in a patient with AFib hereditary amyloidosis. Amyloid deposits are present in the spleen (solid blue arrow) and kidneys (red dotted arrow) (seen more clearly on the posterior scan); **b)** Anterior and posterior whole body scan in a patient with AL amyloidosis. Amyloid deposits are present in the liver (red dotted arrow) and the spleen (solid blue arrow). There is also uptake in the bones which is rarely seen in other types of systemic amyloidosis. The large amyloid deposits in the liver obscure the visualization of kidneys and adrenal glands on planar imaging.[82]

a)



b)



Scintigraphic estimation of whole body amyloid load can provide information on prognosis.[83] AL amyloidosis patients with a large amyloid load have a higher risk of complications associated with chemotherapy, peripheral blood stem cell or solid organ transplantation.[84, 85]

Serial SAP scintigraphy is a useful guide to monitor regression/progression of amyloid deposits - thereby, confirming adequacy of therapy or identifying the need for further therapy. This is useful in all types of systemic amyloidosis including patients with AA,[86] AL,[87] Ab2M,[88] AFib[89] and AApoA1[90] of amyloidosis.

The limitations of ^{123}Iodine labelled SAP scintigraphy include the cost of ^{123}I, availability of SAP (a purified virally inactivated plasma derived component), inability to image diffuse, hollow or small structures such as skin,

gastrointestinal tract or nervous system and most importantly, its inability to image the heart. There are many plausible factors that may account for this major limitation, including movement artefact, ventricular blood-pool content, and possibly the most important factor being a lack of a fenestrated endothelium in the myocardium, hindering access of the large 127kDa SAP molecule to the amyloidotic interstitium within the available timescale of the short half-life of ^{123}I isotope.[80]

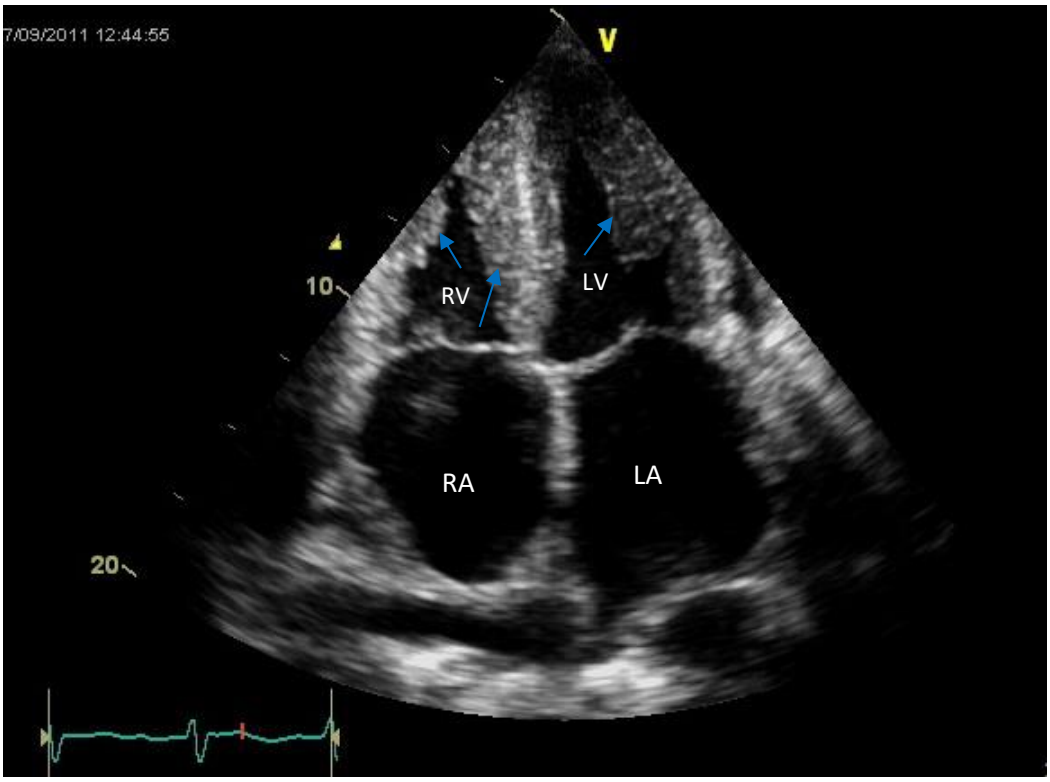
Currently, ^{123}I-SAP-scintigraphy remains the best and only modality in routine clinical use for assessing the extent and distribution of amyloid deposition in all types of systemic amyloidosis.[78] It is part of routine clinical practice at the UK national amyloidosis centre for in-vivo imaging of amyloid deposits. It is also available in the University Medical Centre, Groningen (the Netherlands).

Echocardiography

Echocardiography has been used for the diagnosis of cardiac amyloidosis over the last few decades. Patients with advanced cardiac amyloidosis have characteristic features (Figure 1.3). Echocardiography has both diagnostic and prognostic significance in established disease; however, early diagnosis is challenging.[91] There are many echocardiographic features in cardiac amyloidosis, including, concentric LV wall thickening with right ventricular involvement, impaired biventricular long-axis function and thickened valves (particularly in wild-type or variant ATTR amyloid).[44] Impaired systolic function is a late feature of the disease and carries a poor prognosis. Right ventricular

dilatation is associated with severe cardiac involvement and a median survival of only four months.[44] One of the main features is diastolic dysfunction on echocardiography which may occur before the development of cardiac symptoms.[92] This is one of the most typical features and maybe present in all patients with evidence of restrictive pattern on Doppler mitral inflow assessment. In isolation, none of these are highly specific. A combination of several features is usually necessary and must be interpreted in the context of clinical and other investigational findings. LV wall thickening together with low electrocardiogram (ECG) voltage is suggestive of an infiltrative cardiomyopathy and amyloidosis should be suspected in patients with such combination. This simple combination is reported to have high sensitivity (72-79%) and specificity (91-100%) for cardiac amyloidosis.[93]

Figure 1.3 Echocardiography in cardiac amyloidosis**.** Two dimensional echocardiography in a patient with advanced cardiac amyloidosis demonstrating thickening of the interventricular septum, left ventricular free wall as well as the right ventricle (solid blue arrow). There is bi-atrial enlargement giving an "owl's-eye" appearance to the images.[82]



Longitudinal strain and strain rate analysis maybe abnormal in early amyloidosis[94] and appear to be more sensitive than tissue Doppler, confirming disproportionate impairment of longitudinal contraction despite apparently preserved fractional shortening.[94] LV longitudinal strain in particular may have a role in evaluating prognosis and response to treatment.[95]

Bisphosphonate bone tracers in amyloid imaging

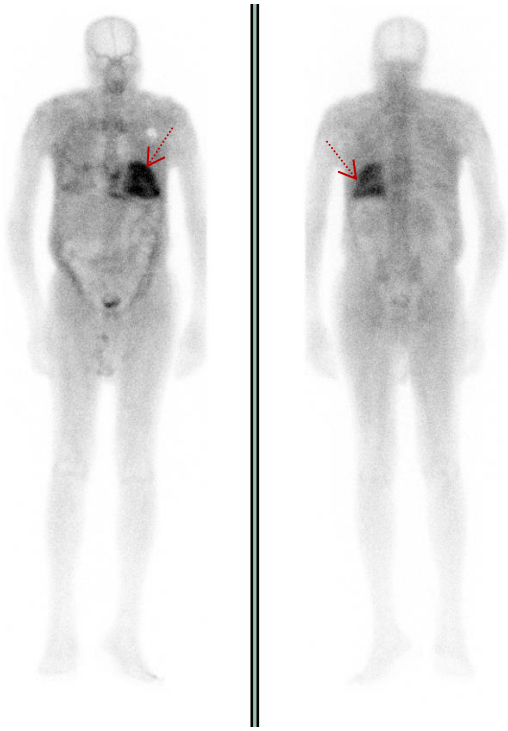
Studies on cardiac amyloidosis imaging using bone seeking radionuclide tracers were inspired following the observation of random myocardial uptake on routine bone scans during the 1970s and 1980s which were later confirmed to be cardiac amyloidosis. A number of tracers have been used, including ^{99m}-technetium-methylene diphosphonate (^{99m}Tc-MDP), ^{99m}-technetium-labeled pyrophosphate (^{99m}Tc-PYP), and ^{99m}Tc-DPD. The exact mechanism of myocardial accumulation of bone-seeking tracers in cardiac amyloidosis is unclear. The use of bone seeking tracers for amyloidosis was largely abandoned in the 1990's due to conflicting results from a number of studies.[96]

The interest in the use of these tracers was recently reignited following a report demonstrating a high sensitivity and specificity of ^{99m}Tc-DPD in imaging cardiac transthyretin amyloid deposits (Figure 1.4). Perugini and colleagues have reported 100% sensitivity and specificity of ^{99m}Tc-DPD in imaging deposits in ATTR variant and ATTRwt.[97] A number of centres[45] have confirmed this finding. ^{99m}Tc-DPD is also taken up in cardiac AL amyloidosis but only in half of the cases with cardiac involvement and the uptake is generally low grade in contrast to the avid high grade uptake in ATTR.

^{99m}Tc-DPD scintigraphy is a valuable, accurate and inexpensive technique allowing non-invasive identification of amyloidotic cardiomyopathy in ATTRwt amyloidosis in elderly patients with unexplained concentric left ventricular hypertrophy (LVH) and a non-dilated LV (heart failure with preserved ejection fraction). Rapezzi *et al* demonstrated that ^{99m}Tc-DPD uptake was seen across a wide spectrum of cardiac involvement ranging from overt cardiomyopathy to cases with normal echocardiograms and normal or near-

normal ECGs raising the intriguing possibility of using ^{99m}Tc-DPD as a screening test for ATTRwt amyloidosis.[98]

Figure 1.4 ^{99m}TcDPD scintigraphy, in cardiac amyloidosis, in a patient with ATTRwt cardiac amyloidosis. There is avid cardiac uptake with marked attenuation of the bone uptake (red dotted arrow).[82]



In summary, ^{99m}TcDPD scintigraphy is a useful and sensitive method of early identification of cardiac ATTR amyloid deposits; possibly at a stage when echocardiography, serum cardiac biomarkers, and perhaps even CMR remain normal.[97] Although the sensitivity of ^{99m}TcDPD scintigraphy opens an immense potential for screening and diagnosis of cardiac ATTR, it is not a diagnostic test

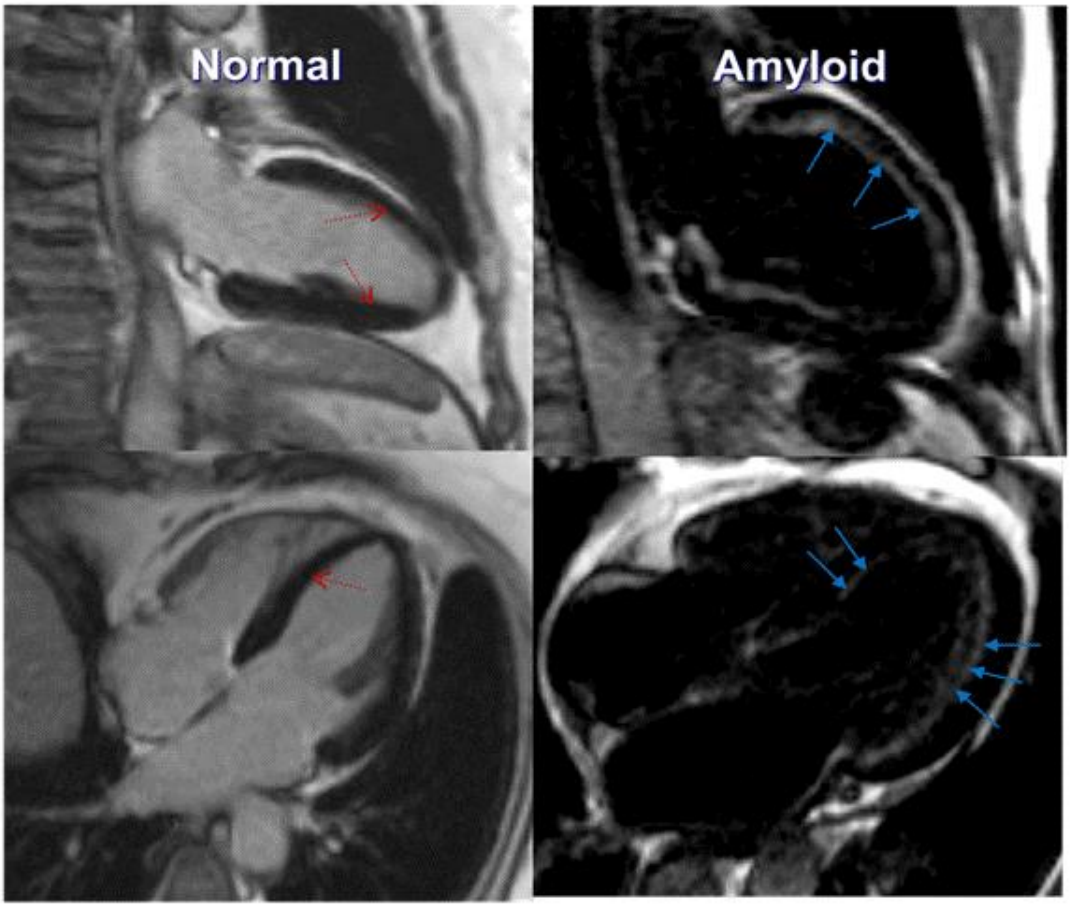
in isolation since uptake of ^{99m}Tc-DPD in the heart occurs in about half of the patients with cardiac AL amyloidosis. ^{99m}TcDPD, therefore, has to be interpreted in context with full assessment of a patient with amyloidosis and integrated with CMR, echocardiography, SAP scintigraphy as well biochemical assessment to increase its specificity and therefore its clinical usefulness.

Cardiac Magnetic Resonance imaging

Cardiovascular magnetic resonance imaging has been found to have a vital role in the diagnosis and prognosis of cardiac amyloidosis.[99] Gadolinium is used as a contrast agent to characterise cardiomyopathies including cardiac amyloidosis. Extracellular space of the myocardium is hugely expanded due to amyloid fibril deposition into which gadolinium is distributed. This results in abnormal kinetics of gadolinium and has been exploited for diagnostic use in amyloidosis (Figure 1.5). Global and subendocardial late gadolinium enhancement occurs after gadolinium contrast injection in cardiac amyloidosis and has been correlated with histological proof of amyloid deposition in the heart.[99] These features are observed in up to 80% of biopsy proven amyloid cases and are reported to correlate with prognosis.[99] CMR can provide better morphological information on cardiac amyloidosis and accurately define systolic function than echocardiography. CMR is especially valuable when echocardiography is unhelpful in the presence of other “hypertrophic” conditions of the heart 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 usefulness of CMR in serial monitoring remains to be established.

Equilibrium contrast CMR (EQ-CMR) is a quantitative technique where an infusion of gadolinium creates equilibrium between the amount of gadolinium in the myocardial interstitium and the plasma - allowing a numerical estimation of the myocardial interstitial volume. Interstitial space within the heart is expanded by fibrosis in many types of cardiac disease, but, EQ-CMR has recently demonstrated a higher extracellular myocardial volume in cardiac amyloidosis than in any other cardiac disease.[100] EQ-CMR may detect amyloid infiltration earlier than conventional magnetic resonance imaging (MRI) and can potentially provide a direct measure of the amyloid burden with scope for use in early diagnosis and disease monitoring.[100]

Figure 1.5 Cardiac magnetic resonance images with late gadolinium enhancement in long axis (vertical) (top) and short axis (horizontal planes) (bottom) in a normal subject and a patient with cardiac AL amyloidosis. In a normal subject (left), signal from the myocardium is nulled and it appears black (solid red line) without areas of white contrast that would indicate myocardial fibrosis or amyloidosis. In a patient with cardiac AL amyloidosis (right) using a similar sequence the expanded myocardial extra-cellular volume leads to nulling of both myocardium, blood pool and diffuse subendocardial enhancements (dotted blue line) – which is characteristic of cardiac amyloid infiltration (Images – courtesy of Dr Marianna Fontana and Dr James Moon, Heart Hospital, UCL, London).[82]



Computerised Tomography

Standard computerised tomography (CT) scanning is useful to detect and monitor organomegaly in systemic amyloidosis but CT features are not specific to amyloidosis. CT scanning is important in patients with lymph node involvement (either isolated or as a part of systemic amyloidosis) to document extent of disease and response to treatment. CT is the imaging method of choice in amyloidosis localised to the respiratory tract. It can provide quantitative assessment of airway narrowing in tracheobronchial amyloidosis, at presentation, follow up, and establish extent of disease by identification of any extraluminal manifestations. High resolution CT (HRCT) is useful in diffuse pulmonary amyloidosis to identify and track the disease course in combination with serial pulmonary function tests.

Positron Emission Tomographic in amyloid imaging

Positron emission tomography (PET) has a better resolution than planar whole-body imaging with standard radionuclides and has the advantage of being quantitative. The role of PET radiopharmaceuticals in imaging systemic amyloidosis is limited. Fluorodeoxyglucose (^{18}F-FDG) is a standardised tracer in PET imaging and is widely available with excellent data on quantification. Standard ^{18}F-FDG-PET has been used to detect metabolic activity in amyloidosis. Amyloid deposits are metabolically inert but the infiltrating cells involved in amyloid formation in localised AL or macrophages involved in amyloid regression in both localised and systemic AL amyloidosis may have enough metabolic activity to be detected by ^{18}F-FDG. Case reports and small

studies suggest that patients with localised AL amyloidosis show ^{18}F-FDG uptake at the sites of localised deposits allowing such deposits to be imaged for the first time and may provide a method for monitoring.[101] Another approach has been to use monoclonal antibodies (mAb) to amyloid fibrils labelled with PET tracers. A phase I study using murine IgG1 mAb 11-1F4 labelled with ^{124}Iodine was studied in 18 patients with AL amyloidosis. 50% of the patients showed uptake in liver, lymph nodes, bone marrow, intestine, or, spleen (but not kidneys or heart). This is undergoing further studies.[102]

Assessment for clonal disorder

The amyloid fibril proteins in AL amyloidosis are derived from the N-terminal region of monoclonal light chains secreted by clonal B cells, predominantly of plasma cell origin. The light chains are more commonly lambda than kappa and consist of whole or part of the variable (V_L) domain, although occasionally intact light chains are present. Monoclonal intact immunoglobulins (M-Igs) are also expressed in about 50-75% of patients with AL amyloidosis.[83] These are predominantly IgG or IgA type and rarely IgM or IgD type. Patients with clinical features consistent with AL amyloidosis should undergo appropriate screening including serum and urine immunofixation and serum free light chain (SFLC).[103] Most patients will have evidence for monoclonal light chain production in the serum, urine, or bone marrow. In 14% of patients with AL amyloidosis, the underlying gammopathy cannot be characterised.

Serum and urine protein electrophoresis and Immunofixation

Serum electrophoresis (SPE) is the conventional means of identifying monoclonal immunoglobulin. This method fails to identify the circulating M-protein in more than half of all patients with AL amyloidosis at the time of presentation.[27] In others, the levels are so low that quantification is either inaccurate or unattainable. This is particularly relevant in the initial detection of the IgD paraprotein which can be challenging as laboratory analysis of IgD-related PCD by SPE usually demonstrates a minimally detectable M-protein spike, often in the β, γ, or β-γ region. A large percentage of cases can actually show hypogammaglobulinemia or a normal serum electrophoretic pattern making detection of the paraprotein difficult.[104] This can apparently occur despite very high levels of IgD in the patient's serum, with some cases mistakenly diagnosed solely as light chain disease.[105] Therefore, it is vital that all patients with what appears to be a light chain only secreting PCD, have IgD paraprotein excluded.

Immunofixation is more sensitive and a monoclonal component is evident in the serum and urine (Bence Jones proteinuria) of 65% and 86% patients respectively using this method but the results are not quantitative and are dependent on renal function.[106] Urine total protein electrophoresis is reported to have a significantly higher detection rate of the IgD myeloma paraproteins than SPE, with up to 96% of cases showing a detectable paraprotein in one study.[104] Thereby, reinforcing the need for both serum and urine protein electrophoretic analysis as standard parallel testing in all patients with suspected AL amyloidosis.

Serum free light chain estimation

The SFLC assay is highly sensitive and quantitative nephelometric immunoassay.[107] It enables the circulating fibril precursor protein in AL amyloidosis to be quantified at diagnosis and also to monitor disease progression or response to treatment in most patients. Monoclonal immunoglobulin light chains are identifiable in 98% of patients with systemic AL amyloidosis using this method. The assay, however, is not specific for AL amyloidosis as monoclonal SFLCs are also found in other B-cell clonal disorders such as MM and monoclonal gammopathy of undetermined significance (MGUS).

As SFLCs are filtered by the glomerulus, the half-life of both kappa and lambda SFLCs is markedly prolonged in patients in chronic kidney disease with the absolute SFLC concentration increasing 20–fold. The range for a normal SFLC ratio therefore alters with progressive renal failure.[108] The ratio of the serum concentrations of the two light chain isotypes rather than their absolute concentrations should be assessed when the glomerular filtration rate is reduced.[109, 110] In addition, the monoclonal component is estimated using the difference between the amyloidogenic and uninvolved SFLC concentration (dFLC) and is applicable to patients with renal failure.[111, 112]

The identification of amyloidogenic light chains cannot rely on a single test and requires the combination of a commercially available SFLC assay with immunofixation of both serum and urine. The association of both techniques has 100% sensitivity.[113, 114] Bone marrow examination usually demonstrates clonal B cells as the source of the light chain production.[115]

Bone marrow aspirate and trephine biopsy

A bone marrow biopsy is mandatory to assess the plasma cell burden[115] and exclude MM and other, less common disorders that can be associated with AL amyloidosis, such as Waldenstrom's macroglobulinemia.[116] The tumour burden in AL amyloidosis is low. About 80% of patients have 'benign' monoclonal gammopathies with average bone marrow plasma cell infiltration of around 7%.[117, 118] Fifteen percent of patients have MM and a smaller proportion have other B cell disorders such as lymphomas. Half of all amyloidogenic PC clones produce light chains only. The lambda clones dominate kappa ones by 4:1, unlike the 2:3 ratio in MM. In addition to routine Congo red staining for amyloid, immunophenotyping is performed on the trephine, to establish clonality.

Multicolour flow cytometry and Cytogenetic analysis

Multicolour flow cytometry (MFC) is more sophisticated technique increasingly being performed on bone marrow samples of AL patients. MFC identifies proportion of normal and clonal PCs. Monoclonal plasma cells are detectable in 97% of patients by flow cytometry immunophenotyping. One study showed that quantification of bone marrow plasma cells (BMPCs) by MFC was a significant prognostic factor for overall survival and in the same study, detecting persistent normal PCs at diagnosis identified a subgroup of patients with AL with prolonged OS.[119] Therefore, MFC immunophenotyping could be clinically useful for the demonstration of PC clonality and for the prognostication of patients with AL.[119]

Assessment of prognostic factors

In addition to the presence and extent of cardiac involvement,[73] high circulating levels of SFLCs were recently shown to be associated with poor outcome.[120] Kumar *et al* have since incorporated SFLC levels at presentation in the Mayo staging system but this is yet to be validated.[121]

Other factors which have been associated with prognosis but have not been integrated into similar staging system include supine systolic blood pressure, the characteristic of the plasma cell clone including percentage of bone marrow plasma cells, the number of organs involved, serum uric acid level, age, serum albumin and performance status.[62, 122]

Identification of a neoplastic plasma cell population adversely affects survival[123], and bone marrow plasma cell infiltration above 10% has also been associated with poorer outcome.[124] Abnormal fluorescence in situ hybridisation (FISH) results at diagnosis has been reported to be prognostic for poorer survival and advanced cardiac disease. Particularly, trisomies and t(11;14) affect survival when degree of plasma cell burden is considered.[125]

Refined imaging techniques, in particular Doppler myocardial strain and strain rate, identify high-risk patients more accurately than standard echocardiographic parameters, adding prognostic information to that derived from cardiac biomarkers.[126] A large whole body amyloid load on SAP scintigraphy and evidence of accumulation of amyloid on serial SAP scans are also poor prognostic factors.[83]

The most favourable prognostic factor is achieving an organ response which is dependent on gaining a deep haematological response.[127] Because of

the frequent time lag in organ response, hematologic response has become an important prognostic measure.[128]

Approach to Treatment

Effective management of AL amyloidosis is challenging due to a combination of the inherent nature of the disease and treatment related complications. Therefore a multidisciplinary approach is essential for the prospect of optimal outcome. Early treatment is associated with improved survival.[83]

All current strategies to manage AL amyloidosis involve systemic therapies designed to destroy the plasma cell responsible for the synthesis of the immunoglobulin light chain.[129] Consequently, all such therapies have been derived from the encouraging results obtained in similar MM populations. AL patients have distinctive organ dysfunction resulting in increased toxicity associated with systemic therapy making both the treatment and response assessment more challenging.

Measuring response to treatment

The efficacy of a treatment can be measured both in terms of reduction in the burden of clonal plasma cell disease (hematologic response) and by improvement in the organ function (organ response).[69] Consensus criteria for hematologic and organ response have been recently developed and validated.[130]

Paraproteins are measurable only in about a quarter of AL amyloidosis patients for monitoring purposes,[83] making conventional immunochemical techniques insufficiently sensitive. Quantification of SFLC allows direct measurement of the amyloidogenic precursor, providing a powerful means for hematologic response assessment.[107] Moreover, SFLC are more powerful predictor of survival in AL amyloidosis than intact immunoglobulins.[110] The degree of SFLC reduction directly correlates with prolonged survival.[120] Therefore, the therapeutic goal is to achieve a deep SFLC response. Stable hematologic disease despite therapy is likely to result in continued effects of the toxic light chain. In 10–15% of patients, the SFLC is only minimally abnormal, therefore, in these patients, monitoring haematological response relies on there being a measurable M-protein, which has been defined as >5 g/l.[131] A minority of patients lack an adequate measurable marker of haematological response.

Organ response

Organ improvement may occur in those who achieve at least a partial hematologic response, with kidney and liver responses occurring most commonly. Organ responses can lag 6 to 12 months behind the hematologic response, necessitating aggressive supportive care and collaborative management with other specialists, particularly in patients with advanced cardiac or renal involvement.

Types of treatment

Alkylators and steroids

Chemotherapy for AL amyloidosis using alkylating cytotoxic agents was described in 1972 with oral melphalan and prednisone (MP) being demonstrated to be the first effective treatment,[132] but the hematologic response rates were not only low but delayed, allowing organ dysfunction to progress in the interim.

Pulsed dexamethasone has also been shown to be active in AL amyloidosis but cause considerable toxicity, most commonly dose limiting fluid retention in patients with nephrotic syndrome and heart failure.[133] Using a low dose, low frequency dexamethasone regimen minimises toxicity whilst providing similar response rates.[134] This activity of single agent dexamethasone led to studies of it in combination with melphalan (MDex), which showed much higher hematologic response rates than MP, and underscores the continued use of MDex in patients who are not eligible for stem cell transplantation (SCT).[135-138]

Novel agents

Thalidomide

Thalidomide, an immunomodulator (IMiD), was the first novel agent explored in AL amyloidosis due to its proven efficacy in MM. As a single agent, thalidomide has limited efficacy.[139] The combination of thalidomide and dexamethasone (ThalDex) is effective, but confers substantial toxicity resulting in poor tolerance and subsequent limited organ response.[140, 141]

ThalDex with cyclophosphamide, (CTD) has been shown to result in a high hematologic response rate.[142] CTD is stem cell sparing, supporting use of this regimen in younger patients whom SCT might subsequently be considered. Nonetheless, the adverse effects of thalidomide, particularly neuropathy, bradycardia, and worsening congestive heart failure, remain problematic and dose limiting in many patients.

Lenalidomide

Lenalidomide is a second generation immunomodulatory (IMiD) agent that has been combined with dexamethasone (LenDex) in treatment of AL amyloidosis.[143-145] LenDex have been combined with either melphalan (MRD)[146, 147] or cyclophosphamide (CRD)[148] but myelosuppression may be a limiting factor.

The non-hematologic toxicity is greater than reported in MM trials (serious adverse events (SAE) 60-86%). The most common adverse effects are cytopenias, rash, fatigue and muscle cramps. Like thalidomide, lenalidomide is prothrombotic, particularly in combination with corticosteroids, therefore, require anti-thrombotic prophylaxis.[149]

Pomalidomide

Pomalidomide is the newest IMiD and is structurally similar to both thalidomide and lenalidomide. Pomalidomide and dexamethasone is a promising therapy for AL amyloidosis.[150, 151]

Bortezomib

Bortezomib is a reversible proteasome inhibitor that triggers stress-activated protein kinases and mitochondrial apoptotic signalling in plasma cells.[152]

Bortezomib with or without dexamethasone has been reported to have 71% hematologic response with 25% complete response (CR) (47% CRs in previously untreated patients) in one study. Notably, cardiac response was documented in 29% of patients, and the 1-year survival rate was 76%.[153]

Cyclophosphamide, bortezomib and dexamethasone (CyBorD) demonstrated significant activity in AL amyloidosis with hematologic responses in 93% of untreated and relapsed patients with 71% achieving CR and patients originally not eligible for stem cell transplantation becoming eligible.[154]

The most common non-hematologic toxicities reported with bortezomib are fatigue, peripheral sensory neuropathy, exacerbation of orthostatic hypotension, peripheral oedema, and constipation or diarrhoea.[155] The incidence of any grade of neuropathy is less than 5% when bortezomib is administered subcutaneously, similar to that observed with weekly intravenous administration.[156]

Bortezomib is rapidly active in AL amyloidosis with high rates of hematologic and organ responses.[154, 157, 158] Table 1.3 shows the clinical studies in AL amyloidosis.

Table 1.3 Clinical studies of conventional and novel chemotherapy agents in AL amyloidosis. [21]

Regimen (Reference)	Clonal response (%)	Overall Survival (months)	Toxicity (>grade 3)	TRM
Melphalan				
MPC [159]	ns	10.6	Non-significant	Nil
MP or MPC [160]	28%	18	Non-significant	ns
Melphalan dexamethasone [135]	67%	Not reached	11%	Nil
Intermediate dose melphalan (IDM) [138]	54%	44	ns	12%
Thalidomide				
Thalidomide (standard dose) [139]	25%	Non-significant	50%	ns
Thalidomide dexamethasone [141]	48%	Non-significant	65%	ns
CTD [142]	74%	Not reached	32%	4%
Lenalidomide				
Lenalidomide ± dexamethasone [143]	75%	Non-significant	73%	Nil
Lenalidomide ± dexamethasone [144]	67%	Non-significant	35%	Nil
CRD [148]	63%	37	74%	9%
CRD [161]	62%	36	57%	Nil
MRD [146]	53%	54% at 2 years	81%	Nil
MRD [147]	44%	24	88%	13%
Pomalidomide				
Pomalidomide ± dexamethasone [152]	48%	24	30%	3%
Bortezomib				
Bortezomib ± dexamethasone [162]	77%	22	ns	Nil
Bortezomib [153]	94%	Not reached	11%	Nil
CyBorD [163]	94%	-	12%	-

High dose melphalan therapy – Autologous Stem cell Transplantation

The introduction of SCT in 1990's was a major breakthrough in the treatment of AL amyloidosis since it held the promise of very rapid and deep haematological responses. Since then, high rates of hematologic and organ response have been documented at many centres, with long-term data among 800 patients demonstrating median survival of over a decade for SCT patients who achieve a CR.[25, 164, 165]

Amyloid-related organ disease[25] and quality of life[166] has been shown to improve in most patients who achieve a CR after SCT. However, SCT carries substantial risks, with high treatment-related mortality reported in early studies.[167] Some centres reported treatment related mortality (TRM) exceeding 40%[128] or more in those with cardiac involvement,[168] reflecting high risks in populations that are not carefully selected.[85, 169] Deaths have also been reported during stem cell mobilisation, reflecting the susceptibility of these patients to unanticipated adverse events such as dramatic fluid retention and pulmonary oedema.[168, 170]

Cardiac staging has helped to minimise TRM by identifying patients susceptible to complications of SCT. Several studies have demonstrated that Stage III patients should be excluded from SCT studies.[171] Incorporating refined selection criteria, TRM can be reduced from 40 to 4%.[170, 172]

SCT should remain a preferred option for patients deemed eligible to undergo this procedure safely. Unfortunately, only a minority (20-25%) of

patients with AL amyloidosis would be eligible for safe SCT. In practice, most patients require alternatives to high-dose therapy.

Post-stem cell transplant consolidation therapy

High-dose therapy does not preclude the use of highly active novel agents in those who do not achieve an adequate response. Adjuvant therapy post–SCT improves haematological response in patients not achieving a CR. This is particularly useful in those with poor response due to risk-adapted SCT.[173-175]

Novel therapies in development

Developments in treatment of AL amyloidosis have continued to follow those of MM, aiming to reduce the burden of underlying clonal cells. In AL amyloidosis, the light chain protein product of the plasma cells causes the disease, and is therefore a separate rational target for therapy.

An alternative approach comprises the combination of a small molecule that depletes circulating SAP co-administered with a monoclonal antibody that can then target SAP associated with amyloid deposits. The novel compound CPHPC ((R) -1-[6-[(R)-2- Carboxy-pyrrolidin-1yl]-6-oxo-hexanoyl] pyrrolidine-2 carboxylic acid) cross-links pairs of SAP molecules in the plasma, triggering their rapid and almost complete removal by the liver.[176] Whilst sustained depletion of circulating SAP is well tolerated and may itself be therapeutic with prolonged administration[177, 178] this treatment does not deplete SAP from amyloid deposits in the very short term. This phenomenon enables the

targeting of residual amyloid-associated SAP with anti-SAP antibodies.[179] A phase I clinical trial recently reported that treatment with CPHPC followed by an anti-SAP antibody safely triggered clearance of amyloid deposits from the liver and some other tissues.[180] Given the universal presence of SAP in amyloid, this combination therapy is potentially applicable to all types of amyloidoses.

These various developments open up the possibility that treatment for AL amyloidosis in the future may involve a combination of novel approaches to inhibit amyloidogenic light chain production in conjunction with therapies that enhance clearance of existing amyloid deposits.

Supportive therapy

Patients with renal amyloidosis are usually nephrotic and therefore are hypoalbuminemic, with consequent oedema. The mainstay of management is diuretics with occasional patients benefiting from albumin infusions. Care must be taken not to prescribe overly aggressive diuretic therapy as it can result in hypotension, syncope, and reduced renal blood flow with a rise in creatinine. Dangerous electrolyte imbalance can also complicate aggressive diuresis.

The management of heart failure often also requires diuretic therapy and hemodynamic stabilisation. Caution is required in the use of standard heart failure medications in patients with amyloidosis. Angiotensin converting enzyme inhibitors and angiotensin receptor blockers are generally not well tolerated as can induce severe hypotension therefore are best avoided. The impact of β blockers and calcium channel blockers on heart rate and myocardial contractility can exacerbate hypotension and heart failure.

Arrhythmias have been reportedly treated with prophylactic amiodarone and have been incorporated into therapy trials of amyloidosis to reduce the risk of sudden cardiac death if complex ventricular arrhythmias are detected on Holter ECG.[135] Caution needs to be exercised with the use of digoxin in cardiac AL patients as they can be exquisitely sensitive to AV nodal blockage and development of digitalis toxicity. Implantable cardiac defibrillators have been used in patients with cardiac involvement because of the high incidence of sudden death, but strong evidence demonstrating their efficacy in this disease is lacking.[181] Alpha agonists such as midodrine can improve orthostatic hypotension due to autonomic neuropathy.

Organ Transplantation

Both cardiac and renal transplantation have been successfully carried out in AL amyloidosis.[182-185] Positive outcomes require strict control of amyloid precursor protein production or recurrence amyloid deposition in the graft is inevitable.

Aims and Objectives

AL amyloidosis is a rare and potentially devastating disease that is possibly under diagnosed and diagnosis is typically delayed. Advances in diagnostic techniques and the use of cardiac biomarkers for staging and free light chains to grade response to treatment have improved care. Nonetheless, patients and the clinicians managing these patients continue to face many challenges. Much of the work within this thesis seeks to explore and understand these challenges. In addition, it looks at possible risk stratifying methods based on plasma cell phenotype and serum clonal markers at presentation, which may be useful in guiding management strategies to improve outcome in AL amyloidosis. And finally, it examines the effectiveness of the currently widely used novel agent, bortezomib.

AL amyloidosis is increasingly recognized in the elderly, mirroring monoclonal gammopathy but very little has been reported on this subgroup of patients where co-morbidities and frailty may compound morbidity and mortality. Moreover, the treatment for AL amyloidosis has to be highly individualised based on age, organ dysfunction, and regimen toxicities. Chapter three focuses on this challenging subgroup of patients. In this chapter, the clinical features, treatment and outcomes in patients over the age of 75 years with systemic AL amyloidosis are analysed.

The next two results chapters concentrate on the rare subtypes, IgM and IgD-related AL amyloidosis. Chapter four focuses on the even rarer subtype, IgD paraprotein-associated AL amyloidosis. The clinical phenotype

and outcomes are uncertain in this cohort of patients. This chapter seeks to improve the understanding of the clinical features and outcomes of patients with IgD related AL amyloidosis.

IgM-related AL amyloidosis, accounting for 6-10% of all AL cases, is a rare and poorly studied clinical entity. Its natural history and management is not clearly defined. Prognostic and response criteria for AL in general have not been validated in this population. Chapter five explores and compares the clinical features, haematological response and overall survival of patients with IgM-related AL amyloidosis in three European countries to that of non-IgM AL patients. The staging and response criteria currently used in non-IgM AL patients are applied and their utility evaluated in the IgM-related AL patients with a view to identifying more specific staging and response criteria in the latter group.

Chapters six, seven and eight examine the potential novel investigations and prognostic markers in AL amyloidosis.

Imaging modalities to detect and delineate soft tissue and lymph node amyloid deposits have not been very well established. Hence, diagnosis is usually based on biopsy of the suspicious lesion if this is deemed safe but histology alone does not provide information on the extent and distribution of amyloid deposits. In chapter four, the role of the bisphosphonate bone tracer, ^{99m}Tc-DPD in detecting amyloid deposits in soft tissue, lymph nodes and lung parenchyma are explored.

Cardiac involvement and presenting dFLC are independent predictors of outcome. However, these markers have less predictive value in patients surviving the initial few months following diagnosis and markers determining

longer term outcomes are needed. The role of plasma cell (PC) clones in determining prognosis has been of recent interest. Multiparameter flow cytometry (MFC) identifies proportion of normal and clonal PCs. Chapter five examines the impact of 'normal' plasma cells, as determined by multicolour flow cytometry, on the outcome of AL patients in the context of the total plasma cell burden as determined by standard morphological techniques. Chapter six explores the utility of a novel method heavy and light chain (HLC) immunoassay, to measure immunoparesis, as an important marker of prognosis in newly diagnosed patients with AL amyloidosis.

Whilst multi-organ failure makes AL patients particularly susceptible to treatment toxicity, the reductions in the concentration of the circulating free light chain (FLC) can rapidly result in marked clinical improvement and prolonged survival. The final results chapter focuses on the treatment of AL amyloidosis. Therapeutic options in AL have broadened in the last decade, mirroring that of multiple myeloma resulting in improved quality of life and extended survival in majority of patients with AL amyloidosis. The combination of cyclophosphamide, bortezomib and dexamethasone (CyBorD) is one of the most commonly prescribed regimens in AL amyloidosis with high rates of hematologic response. However, CyBorD does not overcome the poor prognosis of advanced cardiac amyloidosis. This chapter seeks to identify patients who benefit most from this regimen. The overall haematological response and organ response are analysed. The haematological responses are then determined according to the three Mayo stages.

Chapter Two: Materials and Methods

Declaration

I have designed the studies, collected and analysed the data in my role as a clinical research fellow at the National Amyloidosis Centre, University College London (Royal Free Campus). This thesis comprises of seven studies, of which three are collaborative studies. Chapter five, the IgM-related AL amyloidosis study was in collaboration with the Amyloidosis centres in Pavia, Italy and Limoges, France. Chapter nine, the Bortezomib study was also in collaboration with the Amyloidosis centre in Pavia, Italy. Chapter seven, multicolour flow cytometry study was in collaboration with the Haematological Malignancy diagnostic service, St James's University Hospital in Leeds. The data for the Italian patients in chapters five and nine were provided by Dr Giovanni Palladini, from the Amyloidosis Research and Treatment Centre, Fondazione IRCCS Policlinico San Matteo and Department of Molecular Medicine, University of Pavia, Pavia, Italy. The data for the French patients in chapter five were provided by Dr Murielle Roussel, Department of Haematology, CHU Purpan, Toulouse, France.

Several diagnostic methods were performed by other individuals based at the following sites:

- National Amyloidosis Centre:

- Frozen serum blood samples for the NT-proBNP and Troponin assays for missing data were collected by Wendy Taylor and Lois Cook.
- Histological and immunohistochemical analyses were performed by Janet Gilbertson and Karen Boniface.
- Gene sequencing was performed by Dorota Rowczenio and Hadija Trojer.
- Echocardiography was performed by Babita Pawarova, Oliver Manalo and Sevda Ozer.
- ^{123}I-SAP scintigraphy was performed by Dorothea Gopaul, David Hutt and McKnight.
- ^{99m}TcDPD scintigraphy was performed by David Hutt and Stephanie McKnight.
- Multicolour flow cytometry on bone marrow aspirates of the patients in chapter seven were performed by Anna Baginska, when the bone marrow biopsies were carried out at the National Amyloidosis Centre.

- Royal Free Hospital:
  - Royal Free Hospital laboratory services carried out the serum and urine biochemical investigations and performed measurements for haematological data.
  - Bone marrow trephine analysis for the patients in chapter seven was performed by Royal free Histology department, when the

bone marrow biopsies were carried out at the National Amyloidosis Centre.

- ^{99m}TcDPD scintigraphy was reported by Anne-Marie Quigley, Consultant in Nuclear medicine.

- St James's University Hospital in Leeds
  - Multicolour flow cytometry on bone marrow aspirates and histological analysis of bone marrow trephine for the patients in chapter seven were performed by the Haematological Malignancy diagnostic service, St James's University Hospital in Leeds, overseen by Dr Roger Owen.

Statistics advice was given by Catherine Klersy, from Servizio di Biometria e Statistica, Direzione Scientifica, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, for chapter five.

Patients

All of the patients, (apart from those from international collaborative studies) whose individual details are used in this thesis, have been seen at the UK National Amyloidosis Centre. Chapters five and nine were international collaborative studies. Eighty one of the patients included in chapter five and one hundred and eighteen patients included in chapter nine were seen at Amyloidosis Research and Treatment Center in Pavia, Italy. Thirty one patients included in chapter five were seen at the Amyloid centre in Limoges, France.

The National Amyloidosis centre maintains an Access database with details of all patients found to have amyloidosis which was available for me to utilise. Data on patient deaths were updated on the database based on information from the Office of National Statistics and deceased patients' family members. All patients included in this thesis provided explicit informed consent.

All patients underwent 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.

Functional assessment

Function assessment of patients attending clinic were assessed based on their performance status and heart failure symptoms. Performance status was measured according to the Eastern Cooperative Oncology Group (ECOG) criteria (Table 2.1).[186] This is one of the most widely used scales to assess how the disease affects the daily living abilities of cancer patients, and is a central factor in determining appropriate treatment and prognosis. This criteria has also been used in patients with systemic AL amyloidosis.[187] Heart failure symptoms were assessed using the New York heart association functional classification (NYHA) (Table 2.2).[188]

Table 2.1 Classification of Eastern Cooperative Oncology Group performance status[186]

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 Definition of New York heart association functional classification[188]

NYHA Class	Summary	Description
I	Normal	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (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 dyspnea
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

Histology

Congo Red Staining

Tissues were processed using Puchtler's alkaline alcoholic Congo red method.[189] Rehydrated, formalin fixed, de-paraffinised tissue sections measuring 6-8µg were counterstained with Mayer's haematoxylin under running tap water. Slides were then placed in ethyl alcohol before being stained in fresh Congo Red Working Stain solution. Slides were then rinsed, dehydrated and cleared before sections were mounted in DPX mounting medium. Stained sections were observed in bright-field, and cross polarised light microscopy using a 10x objective. Positive controls, from a known Congo-red positive block validated by laser micro dissection and mass-spectrometry based proteomic analysis were processed in parallel.

Immunohistochemistry

Once the presence of amyloid deposits were confirmed, a panel of monospecific antibodies against known amyloid-forming proteins were used to identify the amyloid fibril. Twenty-two serial sections from each biopsy were cut where possible. Sections were cut into 2µm and 6µm thickness for immunohistochemistry and Congo Red overlay[190] respectively. Immunohistochemistry was carried out using the Sequenza™ (Thermo Shandon, UK) system and antibodies were labelled using Impress™ detection

kits (Vector Laboratories UK) following the manufacturer's recommendations. A metal-enhanced DAB Substrate kit (Thermo Scientific) was used for visualising the immuno-compound. After completing the immunohistochemistry, a Congo red method was performed over the top of the immunostain.[190]

Antibodies routinely used were: AA (Euro Diagnostica), AL lambda, AL kappa, P component, Lysozyme, and transthyretin (DAKO), fibrinogen Aα chain (Calbiochem) and Apolipoprotein AI (Genzyme Diagnostics). All biopsies were also stained with anti-amyloid P component (AP) so that a comparison can be made with that of a negative Congo red. A positive control section for each antibody used was included in every run.

Interpretation of all stained slides were carried out blindly by two experienced workers independently. When the immunohistochemistry is non-diagnostic of amyloid fibril type, laser microdissection of amyloid and mass spectrometry[66] were performed in tandem.

Gene sequencing

Genetic sequencing of the genes implicated in hereditary amyloidosis were carried out when appropriate. Where indicated, whole blood collected in an EDTA tube was frozen and stored for gene sequencing. Genomic DNA was isolated by a rapid method. The coding regions for Apolipoprotein AI (exons 3 and 4), Fibrinogen A α-chain (the 5' end of exon 5) and Transthyretin (exons 2, 3 and 4) were amplified using PuReTaq 'Ready-To-Go' polymerase chain reaction (PCR) Beads (GE Healthcare) with the use of primers as listed in Table 2.3.

Table 2.3 Primers Used in the PCR Process for Genotyping Hereditary Amyloidosis

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

SAP scintigraphy

All patients underwent SAP scintigraphy at the initial visit for the assessment of amyloid deposits in major visceral organs (liver, kidneys, adrenals, bone and spleen) and at subsequent visits if clinically indicated for the monitoring of amyloid deposits.

Female patients between the ages of 12 and 55 years were asked to confirm that they were not pregnant and sign the pregnancy declaration form prior to injection. The need for thyroid blockade and oral dosing schedule of potassium iodide (six doses over three days) were explained to patients. The first dose (60mg) is administered to the patient prior to the injection. Following thyroid blockade, patients received 200μg of SAP with 190MBq of radiolabelled iodine (^{123}I), the equivalent of 3.8mSV of radiation by intravenous bolus injection. Six or 24 hours after injection, anterior and posterior whole-body images and appropriate regional views were obtained with a General Electric Starcam gamma camera (IGE Medical Systems, Slough, UK).

Amyloid load was classified as follows: ‘normal’ - no abnormal localisation of the tracer; ‘small’- uptake in one or more organs visible with normal intensity in the blood pool; ‘moderate’ - abnormal uptake within organs and diminished blood pool; ‘large’ - blood pool signal lost with adjustment of the grey scale to encompass the target organ.

Follow-up scans were performed, when feasible, at approximately yearly intervals. Amyloid progression and regression on these scans were defined as follows: ‘progression’- increase in the tracer uptake within an affected organ or a

reduction in the background blood-pool signal in combination with a stable amyloid burden; ‘regression’ - reduction of the tracer within an affected organ and/or an increase in the background blood pool when compared with the previous scans.

Cardiac assessment

Patients routinely undergo a combination of blood tests (cardiac biomarkers), electrocardiogram, echocardiogram and most recently cardiac MRI (CMR), for assessment of evidence of cardiac amyloidosis. Amyloidotic heart muscle can not to be visualised on ^{123}I-SAP-scintigraphy, due to movement artefact, ventricular blood-pool content, and a lack of a fenestrated endothelium in the myocardium, hindering access of the large 127kDa SAP molecule to the amyloidotic interstitium within the available timescale of the short half-life of ^{123}I isotope.[80]

Cardiac biomarkers and Mayo staging

Measurement of the cardiac biomarkers, are vital part of cardiac assessment and risk stratification in patients with AL amyloidosis. All patients had blood tests at presentation and at each visit. This included the measurement of cardiac biomarkers, N-terminus pro-B Natriuretic Peptide (NT-proBNP) and Troponin which were used to risk stratify patients using the Mayo staging system, defined as follows:[73] Stage I - NT-proBNP <332ng/L and cTnT <0.035mcg/L or Troponin I <0.1ng/mL, stage II – NT-proBNP >332ng/L or cTnT

>0.035mcg/L or Troponin I >0.1ng/mL, and stage III both NT-proBNP >332ng/L and cTnT >0.035mcg/L or Troponin I >0.1ng/mL.[73] In chapter nine, stage III patients were divided in two groups based on whether they had NT-proBNP below (stage IIIa) or above (stage IIIb) 8500ng/L, which is known to be associated with a very poor prognosis.[74]

Blood pressure and 12 lead Electrocardiogram

All patients had lying and standing blood pressure measured by the nursing staff. All patients had a standard 12 lead ECG at presentation and subsequent visits. The ECG was acquired using a calibration of 10 mm/mV and speed of 25 mm/s. The presence of low voltage on 12-lead electrocardiography (ECG) (all limb leads less than 5 mm in height), as defined in the amyloidosis consensus criteria, was considered suspicious of cardiac involvement by amyloid.[69]

Echocardiography

All patients underwent transthoracic echocardiography at presentation and subsequent visits. Parasternal long axis and apical long axis views were most commonly used. Echo studies, included tissue Doppler. Scans were performed and analysed by two echocardiographers experienced in scanning patients with cardiac amyloidosis. Accepted markers of diastolic dysfunction i.e. isovolumic relaxation time (IVRT), E-deceleration time and E:E' ratio were measured.[191] In addition, left ventricular wall thickness, left ventricular systolic function and atrial diameter were measured using defined criteria from the British Society of Echocardiography.

99m-technetium-3,3,-diphosphono-1,2-propanodicarboxylic acid scintigraphy

^{99m}TcDPD scintigraphy is only performed in patients with suspected cardiac amyloidosis. Patients were scanned using two General Electric (GE) 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) after intravenous injection of 700MBq of ^{99m}Tc-DPD. Whole body planar images were acquired 3 hours post-injection followed by cardiac SPECT-CT. The whole body sweep images were acquired using low energy, high-resolution collimators and a scan speed of 10cm/min. SPECT-CT reconstruction and image fusion were performed on the GE Xeleris workstation. The CT raw data were reconstructed three times using soft tissue, lung, and bone settings with a 512 matrix and 3.75mm slice thickness. The soft-tissue reconstruction was loaded into the Myovation programme on the Xeleris to perform the attenuation correction on the SPECT data. The SPECT data were reconstructed using filtered back projection. Data were pre-filtered using a Butterworth filter with a critical frequency of 0.4 cycles/cm and a power of 10. It was then reconstructed with a quantitative ramp filter.

Cardiac retention of ^{99m}Tc-DPD was visually scored using a modification of the grading devised by Perugini *et al*.[97] 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. All scans were reported by two experienced clinicians who were blinded to all clinical data.

Renal staging

In chapters three and nine, staging of renal damage was performed according to recently published criteria.[192] Renal stage was defined by eGFR (cut off 50mL/min per 1.73m^2) and proteinuria (cut off 5g/24h); stage I patients have both eGFR above and proteinuria below the cut off, stage II have either eGFR below or proteinuria above the cut off, and stage III patients have both eGFR below and proteinuria above the cut off.

Criteria for diagnosis of amyloid and definition of organ response

Amyloid organ involvement were defined according to the international consensus criteria (ICC) 2010 along with SAP scintigraphy findings.[70] Cardiac response was assessed as per the consensus criteria published by Palladini *et al*.[130] (Table 2.4)

Table 2.4 - Definition of Organ Involvement and Organ Response[70, 130]

Organ	Definition of organ involvement	Definition of organ response	Definition of disease progression
Kidney	- 24-hr urine protein >0.5g/day, predominantly albumin	- 50% decrease (at least 0.5g/day) of 24-hr urine protein (urine protein must be >0.5g/day pre-treatment) Creatinine and - Creatinine clearance must not worsen by 25% over baseline	- 50% increase (at least 1g/day) of urine protein to greater than 1g/day or - 25% worsening of serum creatinine or creatinine clearance
Heart	- Echo: mean wall thickness >12mm, no other cardiac cause	- NT-proBNP response (>30% and >300ng/L decrease if baseline NT-proBNP ≥650ng/L) - Mean interventricular septal thickness decreased by 2mm - 20% improvement in ejection fraction - Improvement by 2 New York Heart Association classes without an increase in diuretic use, and - No increase in wall thickness	- NT-proBNP progression (>30% and >300ng/L increase) - Interventricular septal thickness increased by 2mm compared with baseline - An increase in New York Heart Association class by 1 grade with a decreasing ejection fraction of >10% - EF progression (10% decrease)
Liver	- Total liver span >15cm in the absence of heart failure or	- 50% decrease in abnormal alkaline phosphatase value	- 50% increase of alkaline phosphatase above the lowest

	- Alkaline phosphatase >1.5 times institutional upper limit of normal or - SAP scintigraphy evidence	- Decrease in liver size radiographically at least 2cm - Regression on SAP scintigraphy	value - Progression on SAP scintigraphy
Peripheral nerve	- Clinical; symmetric lower extremity sensorimotor peripheral neuropathy	- Clinical assessment - Improvement in electromyogram nerve conduction velocity (rare)	- Clinical assessment - Progressive neuropathy by electromyography or - Nerve conduction velocity
Autonomic nerve	- Gastric-emptying disorder, - Pseudo-obstruction, - Voiding dysfunction not related to direct organ infiltration	- Clinical assessment	- Clinical assessment
Gastrointestinal Tract	- Direct biopsy verification with symptoms	- Clinical assessment	- Clinical assessment
Lung	- Direct biopsy verification with symptoms - Interstitial radiographic pattern	- Clinical assessment - Pulmonary function tests	- Clinical assessment - Radiographic evidence
Soft tissue	- Tongue enlargement, - Clinical Arthropathy - Claudication - Presumed vascular amyloid - Skin	- Clinical assessment	- Clinical assessment

	- Myopathy by biopsy or pseudohypertrophy - Lymph node (may be localized) - Carpal tunnel syndrome		
Spleen	- SAP scintigraphy	- Regression on SAP scintigraphy	- Progression on SAP scintigraphy
Adrenal	- SAP scintigraphy	- Regression on SAP scintigraphy	- Progression on SAP scintigraphy

Assessment of clonal disease

Total immunoglobulins, Serum protein electrophoresis and immunofixation electrophoresis

All patients had total immunoglobulin levels measured on a BN^{TM}II System nephelometer (Siemens, Germany). Serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE) (Sebia, France) were carried out using standard laboratory procedures. As, some cases can be difficult to diagnose, e.g. with the low concentration of monoclonal protein that is often typical of IgD-related plasma cell dyscrasia,[105] great care is exercised in the interpretation of electrophoresis patterns and immunoglobulin profiles. All patients seen at the centre, with suspected AL amyloidosis are routinely and specifically screened for the presence of IgD monoclonal protein, especially in cases where no M-protein is detected on SPE and the underlying clonal disorder appears to be of just light chain origin.

Serum free light chain assay

All patients had blood tests for kappa and lambda SFLC at presentation using latex-enhanced immunoassay - (The Binding Site, Birmingham, United Kingdom) on a Behring BNII auto-analyser (Dade Behring, Marburg, Germany).[107] Serial measurement of SFLC were also carried out at monthly intervals during treatment with chemotherapy and 1-2 monthly thereafter. This forms a standard part of the assessments in systemic AL amyloidosis.[110]

The assay utilises antibodies directed against SFLC epitopes that are hidden in whole immunoglobulin molecules, and has a sensitivity of <5mg/l. The reference range was determined by testing the sera from 100 healthy blood donors; the mean concentrations of polyclonal free kappa and free lambda light chains were 11.38mg/L (95% CI, 7.41-16.77mg/L) and 17.36mh/L (95% CI, 8.91-29.87mg/l) respectively. The mean kappa/lambda ratio was 0.70 (95% CI, 0.37-0.95).

Kappa or lambda values that exceeded the respective reference ranges and produced an abnormal kappa to lambda ratio in the context of preserved renal function was considered evidence of an underlying clonal disorder. In patients with renal impairment the ratio alone was used. SFLC values were considered evaluable for assessing response if the pre-treatment dFLC was >50mg/L with an abnormal SFLC ratio. The definitions of haematological response are outlined in Table 2.5.

Table 2.5 - Haematological Response Criteria[130]

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) and 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 and 50% reduction
Non responder	- Patients who could not be classed as achieving SFLC-PR or better
Progression	- From CR, any detectable monoclonal protein or abnormal free light chain ratio (light chain must double) - From PR or stable response, 50% increase in serum M protein to >0.5g/dL or 50% increase in urine M protein to >200 mg/day; a visible peak must be present - Free light chain increase of 50% to >10mg/dL (100mg/L)
Stable	- No CR, no PR, no progression

Haematological response criteria

Hematological responses were assessed as per the consensus criteria published by Palladini *et al.*[130] The responses were assessed as the best achieved response after starting chemotherapy and before any further therapy was given. Those who died early prior to response assessment were categorised as non-responders in the intent to treat analysis (ITT).

Hevylite assay

Hevylite (The Binding Site Group Ltd, Birmingham, UK) are sheep polyclonal antibody-based immunoassays targeted at unique junctional epitopes between the heavy chain and light chain constant region of intact immunoglobulins (heavy and light chain, HLC). The assays allow separate quantification of IgGλ, IgGκ, IgAλ, IgAκ, IgMλ and IgMκ in serum. In patients with monoclonal gammopathies, Hevylite measurements give an indication of the monoclonal and non-clonal immunoglobulin production (e.g. in an IgGλ patient, IgGλ and IgGκ concentrations, respectively). These can be measured in pairs to calculate HLC ratios (e.g. IgGλ/IgGκ); HLC ratios outside the reference range can give a sensitive indication of clonality. Table 2.6 shows the manufacturer's reference ranges for Hevylite immunoassays on the BNII nephelometer.

Table 2.6 - Reference ranges for Hevylite immunoassays on the BNII nephelometer (as provided by manufacturer)

IgGκ	4.03 – 9.78g/L
IgGλ	1.97 – 5.71g/L
IgGκ/IgGλ	0.98 – 2.75
IgAκ	0.48 – 2.82g/L
IgAλ	0.36 – 1.98g/L
IgAκ/IgAλ	0.80 – 2.04
IgMκ	0.29 – 1.82g/L
IgMλ	0.17 – 0.94g/L
IgMκ/IgMλ	0.96 – 2.30

The results from this method were a vital part of chapter eight. HLC concentrations (IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ) using Hevylite® assays (The Binding Site Group Ltd, UK) were measured on serum samples collected at the time of presentation, and stored at -80°C prior to any therapy of the patients in chapter eight.

Hevylite measurements were carried out on a BNII nephelometer (Siemens, Germany) using stored serum samples. Evaluating the concentration of a soluble antigen by nephelometry involves the addition of the test sample to a solution containing the appropriate antibody in a reaction

vessel or cuvette. A beam of light is passed through the cuvette and as the antigen-antibody reaction proceeds, the light passing through the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. The light scatter is monitored by measuring the light intensity at an angle away from incident light. A series of calibrators of known antigen concentration are assayed initially to produce a calibration curve of measured light scatter versus antigen concentration. Samples of unknown antigen concentration can then be assayed and the results read from the calibration curve.

Bone marrow biopsies

All the patients in chapter seven had bone marrow biopsies performed prior to treatment, either at the National Amyloidosis Centre or at their local hospital. When the procedure was done at the centre, the trephine biopsy was sent to Royal Free Histopathology department for further analysis. Bone marrow trephines performed outside of the centre were reviewed at the Haematological Malignancy diagnostic service, St James's University Hospital in Leeds. The plasma cell burden was morphologically estimated on the bone marrow trephine biopsy (BMT) on haematoxylin-eosin stain and by CD138 immunohistochemistry, by an experienced haematopathologist. 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.

Flow cytometry

Flow cytometric analysis was performed at Haematological Malignancy Diagnostic Service, Leeds or the National Amyloidosis Centre (NAC), London, United Kingdom, according to the principles outlined by the European Myeloma Network.[193] Bone marrow biopsies were performed as part of the ongoing AL amyloidosis bone marrow study at the National Amyloidosis Centre. Leukocytes were prepared by incubation of a volume of BM aspirate containing 106 leukocytes with 5 mL of ammonium chloride (8.6g/L in distilled water) for 10 minutes at 37°C, washed twice, and suspended in 5mL of FACS Flow (BD Biosciences, Oxford, United Kingdom) containing 0.3% bovine serum albumin. The cell pellet was re-suspended in pre-titered antibody mixtures and incubated for 30 minutes at 4°C in the dark, washed twice, and re-suspended in FACS Flow. A minimum of 100,000 events were acquired analysed for each antibody combination using a Canto II flow cytometer with FACS Diva software (BD Biosciences). A six-colour panel of antibodies was used: CD138 APC (B-B4; Miltenyi Biotec), CD45 APC-Cy7 (2D1; BD Pharmingen, Oxford, United Kingdom), CD38 PE-Cy7 (HIT2; BD Pharmingen), and CD19 PerCP-Cy5.5 (HIB19; BD Pharmingen) and the eight colour panel included additionally CD81 and CD20. In all cases, expression of CD56 PE (MY31; BD Biosciences) and CD27 FITC (M-T271; BD Pharmingen) on gated plasma cells was assessed. An aberrant phenotype was classified as a lack of CD19 expression, strong CD56 expression, weak CD27 expression, and/or weak CD45 expression and were defined as “abnormal” plasma cells. Plasma cells expressing CD19 and lacking the aberrant phenotype were defined as “normal’’ plasma cells.

Statistical analysis

Statistical analysis was undertaken using the SPSS 21 software package (SPSS, Chicago, IL) in all the studies apart from the two international collaborative studies in chapters five and nine. Stata 13.1 (StataCorp, College Station, TX, USA) was used in chapter five and MedCalc Statistical Software version 14.10.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014) was used in chapter nine. Individual statistical methods are discussed separately in each results chapter.

Results

Chapter Three: Clinical profile and treatment outcome of elderly patients with systemic AL amyloidosis

This chapter is written in the context of my publication:

Clinical profile and treatment outcome of older (>75 years) patients with systemic AL amyloidosis. Sachchithanantham S, Offer M, Venner C, Mahmood SA, Foard D, Rannigan L, Lane T, Gillmore JD, Lachmann HJ, Hawkins PN, Wechalekar AD. Haematologica. 2015 Nov;100(11):1469-76. Copyright permission obtained from Haematologica office for use in my thesis.

Introduction

Historically, systemic AL amyloidosis has been reported to have a very poor prognosis with a median survival of about 13 months[194] but in the last two decades with the adoption of autologous stem cell transplantation and availability of novel therapeutic agents, improved outcomes have been reported with a median survival of 46 months by the Italian group[195] and 3.3 years in the UK[196]. Although, treatment of AL amyloidosis is aimed at the underlying clonal disorder, similar to that of multiple myeloma, patients with AL amyloidosis tend to experience a higher rate of treatment related toxicity due to vital organ involvement. This poses a great challenge in the management of elderly

patients with AL amyloidosis as multiple co-morbidities and frailty further reduce the threshold for developing treatment related toxicity leading to premature termination of treatment or not being considered for therapy at all. Consequently, traditionally, treatment decisions in this group have not been straightforward and require careful risk assessment prior to embarking on a potentially toxic therapy.

Anagnostopoulos reported that elderly (>70 years) patients with multiple myeloma have a significantly shorter survival than their younger counterparts with twice the risk of experiencing early death in the older age group.[197] The increased risk has been attributed to the presence of co-morbidities and higher toxicity from chemotherapy, leading to poor tolerability resulting in early discontinuation of treatment and suboptimal response. Subsequently, guidelines have been established to categorise patients into risk groups and tailor therapies to individual patients.[198]

As the population ages and with 5% prevalence of MGUS in those aged over 70 years[199], the incidence of AL amyloidosis in the older population is likely to increase. There is very little reported on the outcome of this potentially growing subgroup of patients with AL amyloidosis. The toxicity profile of novel therapies are promising and may be better tolerated than some of the conventional therapies, permitting treatment of carefully selected elderly patients with AL amyloidosis.

17% of AL amyloidosis patients seen at the UK NAC are >75 years of age at presentation. The aims of chapter three are to understand the outcome of patients over the age of 75 years with systemic AL amyloidosis, and to explore whether treating elderly patients translates into a better survival and

how it compares to the predicted life expectancy of a 75 and 80 year old person in the UK. The chapter also explores risk stratification models, and studies the impact of treatment on survival whilst characterising the features of patients who received greatest benefit from treatment in terms of survival and improvement in amyloidotic organ function.

Methods

Patient selection

A retrospective review of all the patients above the age of 75 who had been evaluated at the UK NAC between 2005 and 2012 were studied. All patients with AL amyloidosis under the age of 75 years seen during the same study period, were also identified, to derive proportion of older patients and overall survival outcomes.

Outcome measures and Statistics

The primary outcome measures studied were haematological response to treatment and overall survival. The response was assessed as the best achieved response after starting chemotherapy and before any further therapy was given.

Statistical analysis was undertaken using the SPSS 21 software package (SPSS, Chicago, IL). Categorical variables were compared with chi-square or Fisher's exact tests as appropriate. Survival was assessed by the method of

Kaplan and Meier and compared by log-rank test. All *P* values were 2 sided with a significance level of 0.05. Multivariate analysis was by Cox or binary logistic regression as appropriate. All analyses were on intent to treat basis. Two land mark analyses were performed: at six months and two years, the former time point was to evaluate the impact of presenting factors on early survival. Amyloidotic organ responses are frequently delayed and only patients who survive long enough after treatment would benefit from these responses. The latter time point was chosen as this was close to the median survival of the whole cohort and by which time patients would have organ responses and, most importantly, in an elderly population, identifying patients who would genuinely benefit from a response to treatment is important.

Results

A total of 295 patients with AL amyloidosis older than 75 years of age were identified, accounting for 16% of a total of 1870 AL amyloidosis patients reviewed during the study period. The proportion of patients over the age of 75 years increased from 13% in 2005-2006, to 14% in 2007-2008, 17% in 2009-2010 and 19% in 2011-2012.

The presenting characteristics of the patients are detailed in Table 3.1. There was a male predominance (male: female ratio of 1.4:1). 65% of patients had a detectable M-protein. The median paraprotein at presentation was 8g/l (range 1-57). 35% of patients had an isolated light chain clone. The median number of organs involved was 2 (1-7). Among the entire group, there was echocardiographic evidence of cardiac involvement in 51% of cases. Of the

252 patients (85%) with full baseline cardiac biomarkers (both NT-proBNP and cardiac troponin-T) available for staging, 54% had Mayo stage III disease at presentation. Among the remaining 43 patients with an incomplete set of biomarkers at baseline, 88% had abnormal NT-proBNP and 52% had cardiac involvement as defined by echocardiographic criteria. Renal function was normal in only 9% and severe renal impairment (≥stage IV chronic kidney disease (CKD)) was seen in 30% of patients. Ten percent of the cohort had a median systolic blood pressure less than 100mmHg and 24% of the cohort had NT-proBNP concentration ≥8500ng/L, both cut-offs associated with a particularly poor prognosis.[74]

Table 3.1 Baseline characteristics of the whole patient cohort, and the survivors and non-survivors of the two year landmark analysis of treated patients.[200]

Patient characteristics	Entire cohort – Median (range)/ number of patients (%)	Treated patients at 2 years - Median (range)/number of patients (%)		
		Non survivors (n=113)	Survivors (n=108)	*P* value
Age at presentation - 75-80 years - >80 years	78.5 (75-94.3) 205 (69%) 90 (31%)	78.4 (75-94.3)	78.2 (75.1-86)	0.561
Sex (Male: Female ratio)	1.4:1	1.4:1	1.6:1	0.784
Age at death (years)	79.8(75.2-95.2)/181 (61%)	79 (75.7-95.2)	81.6 (78.4-87.8)	
Monoclonal protein type				
IgG	118 (40%)	46 (41%)	39 (36%)	
IgA	39 (13%)	12 (11%)	15 (14%)	
IgM	35 (12%)	14 (12%)	13 (12%)	
Light chain only	102 (35%)	41 (36%)	40 (37%)	
Paraprotein concentration (g/L)	8 (1-57)	8 (1-57)	8 (2-26)	
Involved light chain type				
Kappa (mg/L)	264 (29.1-3880)/ 71 (25%)			
Lambda (mg/L)	168 (26.9-14000)/ 211 (74%)			
Baseline involved FLC (mg/L)	197 (26.9-14000)	240 (27.2-5631)	143 (26.9 – 3880)	0.001
Baseline dFLC (mg/L)	174.2 (0.80 – 13990)	224(12.2-5594.1)	113.6 (7.5-3877.90)	0.002
Organ involvement				
No of organs involved	2 (1-7)			
1 Organ	85 (29%)	18 (16%)	39 (36%)	0.002
2 Organs	106 (36%)	45 (40%)	36 (33%)	
3 or more Organs	105 (35%)	50 (44%)	33 (31%)	
Cardiac involvement	146 (51%)	73 (67%)	42 (39%)	
Systolic blood pressure (mmHg)	126 (71-202),	121 (71-192)	132 (80-181)	0.005
Systolic blood pressure <100 mmHg	31 (10%)	16 (14%)	4 (4%)	0.006
NT-proBNP (ng/L)	2720 (51-112992)	4093 (118.6-50271.2)	843.2 (93.2-70144.0)	<0.0001

NT-proBNP ≥8500ng/L	71 (24%)	33 (29%)	15 (14%)	0.006
cTnT (ng/ml)	0.0515(0.003-0.578)	0.057 (0.00-0.578)	0.0245 (0.00 – 0.269)	<0.0001
Mayo stage*	n = 252 (85%)	n = 98 (87%)	n= 92 (85%)	
I	30 (12%)	3 (3%)	17 (18%)	<0.0001
II	86 (34%)	26 (27%)	45 (49%)	
III	136 (54%)	69 (70%)	30 (33%)	
Renal involvement	230 (78%)	110 (97%)	86 (80%)	0.613
Serum creatinine (µmol/L)	124 (39-1285)	126 (49-1285)	111 (39-612)	0.081
24 Urine protein (g/24h)	3.4 (0-20.0)	2.02 (0-11)	4.48 (0-20)	0.021
eGFR (ml/min)	46 (>90-ESRF)	44 (>90 – ESRF)	51.5 (>90-ESRF)	0.076
≥CKD stage IV	88(30%)	32 (29%)	23 (21%)	0.243
Renal stage				
1	37 (13%)	17 (17%)	18 (21%)	0.844
2	116 (39%)	60 (58%)	39 (46%)	
3	71 (24%)	26 (25%)	27 (32%)	
Liver involvement				
Consensus criteria	42 (15%)	25 (23%)	6 (6%)	0.001
Alkaline phosphates (U/L)	89 (33-1717)	93 (41-1717)	85 (33-902)	0.021
SAP	93 (32%)	41 (36%)	30 (28%)	0.176
Soft tissue	57 (20%)	24 (22%)	21 (19%)	0.665
PNS	29 (10%)	15 (13%)	7 (7%)	0.092
ANS	54 (19%)	26 (23%)	20 (19%)	0.411
GI	35 (12%)	13 (12%)	8 (7%)	0.279
NYHA class				
1 – 2	143 (79%)	47 (70%)	58 (95%)	<0.0001
3-4	39 (21%)	20 (30%)	3 (5%)	
ECOG performance status				
0 – 1	148 (52%)	43 (40%)	76 (72%)	<0.0001
2	95 (33%)	43 (40%)	24 (23%)	
≥ 3	43 (15%)	22 (20%)	5 (5%)	

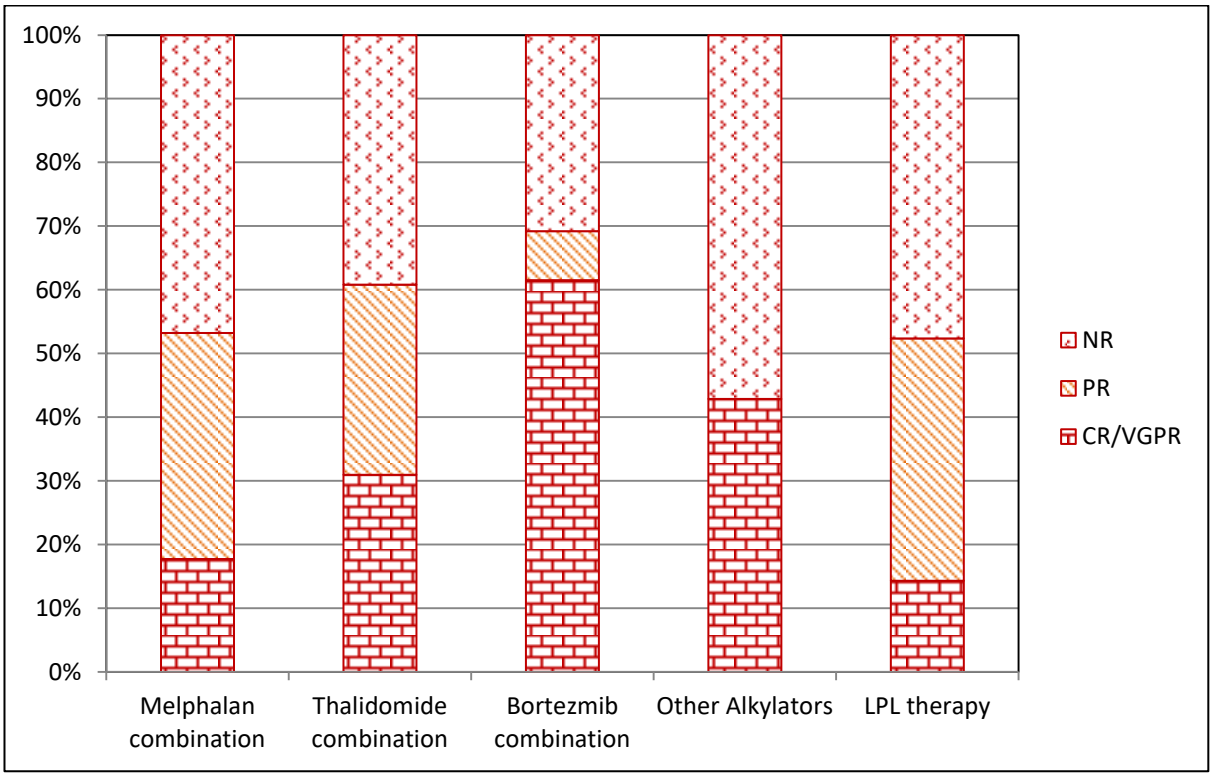
Treatment response

238 (81%) patients were given chemotherapy, and 57 (19%) patients made an informed decision to continue with supportive care only. Details of chemotherapy were incomplete in 19 (8.5%) patients and their data were excluded from the analysis of treated patients. Patients received a median of 4 cycles (range 1-10). Thalidomide based combinations, mostly dose attenuated oral cyclophosphamide, thalidomide and dexamethasone (CTDa), were used in 100 patients (45%), melphalan-dexamethasone were used in 63 (29%), bortezomib based regimens in 30 (13%), other alkylator-steroid combinations in 7 (3%), and various regimens for lymphoplasmacytic lymphoma in 22 (10%) patients. 32% of patients received less than three cycles of treatment and only 67 (35%) patients completed their planned course of six cycles of treatment.

Evaluation of haematological response to treatment was undertaken as ITT analysis of all 284 (96%) patients and a separate ITT analysis of only those patients who actually received chemotherapy. Five patients were excluded as their clonal markers were insufficiently elevated to enable assessment of response, and six patients did not attend follow-up. On an entire cohort ITT analysis, 125 (44%) patients had a haematological response with 64 (23%) patients achieving a CR or VGPR (11% CR and 12% VGPR) and 61 (21%) attaining a PR. On an ITT analysis of 227 patients who received chemotherapy, a haematological response was achieved by 125 (55%) patients with 28% CR/VGPR and 27% PR. One hundred and ninety seven of 238 (83%) treated patients were entered in the evaluable response analysis. This excluded 30

patients who died before response assessment. Of the evaluable patients, 125 (63%) achieved a haematological response consisting of 32% with CR or VGPR (15% and 17% respectively) and 31% with a PR. Sixty nine percent of the patients treated with a bortezomib based regimen attained a haematological response, with 61% achieving a VGPR or better. Fifty three percent of those on melphalan and 61% on thalidomide based regimens achieved haematological responses respectively (Figure 3.1). 21/197 (11%) patients in the 75-80 year group and 9/87(10%) patients in over 80 year age group achieved CR suggesting that age did not appear to substantially impact CR rates.

Figure 3.1 Haematological response achieved with various chemotherapy combinations on an ITT basis.[200] The response rates were: Thalidomide based combinations – CR/VGPR 30(31%), PR 29(30%); Melphalan dexamethasone – CR/VGPR 11(18%), PR 22(35%); Bortezomib based – CR/VGPR 16(61%), PR 2(8%); other Alkylators – CR/VGPR 3(43%), PR 0(0%); regimens for Lymphoplasmacytic lymphoma – CR/VGPR 3(14%), PR 8(38%).



Following appropriate counselling, 57 patients decided not to receive chemotherapy and have supportive care only. The median age at diagnosis for this subgroup was 79 years (22 (39%) were over 80 years of age). The median age at death was 80 years and median survival without treatment was 8 months. These patients had more severe disease than those who received chemotherapy. Cardiac, renal and liver involvement were seen in 25 (46%), 37 (65%) and 19 (33%) patients respectively. 67% of patients had Mayo stage III disease with a median NTproBNP of 6695ng/L of and 42% had NT-proBNP above 8500ng/L. Thirty patients (53%) had eGFR less than 30mls/min at presentation and 11 (20%) had systolic blood pressure (SBP) <100mmHg. Twelve patients (32%) had NYHA class 3 or above and 37 (66%) had ECOG ≥2.

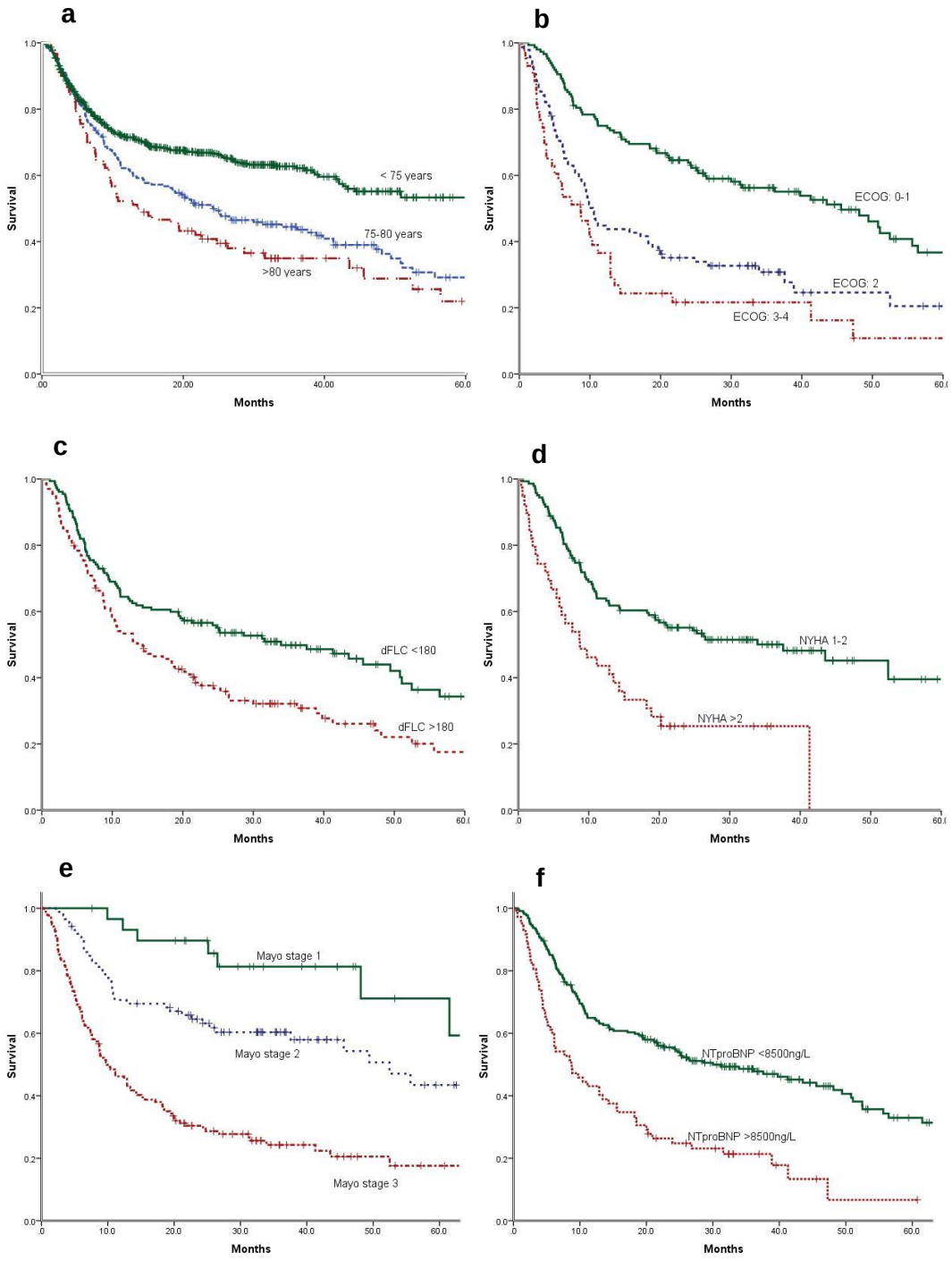
Toxicity data was recorded in detail from 2009 as a part of a prospective observational study (ALCHemy) at the National Amyloidosis Centre. This was available for 147 patients of whom 113 (77%) experienced grade three or greater toxicity. Fluid retention in 32% of patients was the most commonly reported adverse event followed by infection or sepsis in 17%. Thalidomide based regimens were associated with the greatest toxicity (84%) and bortezomib based regimens had the least (70%), but was not statistically significant (Fisher's exact p=0.141).

Survival analysis

The median overall survival (OS) of the entire cohort was 20.9 months and OS at 1, 2 and 5 years were 59%, 47% and 26% respectively, which is inferior to

the outcome for AL amyloidosis patients aged <75 years seen at the NAC during the same period (median OS 6.1 years) (Figure 3.2a). Early deaths at 2, 3 and 6 months were seen in 4%, 9% and 22% of patients respectively. The OS of patients aged between 75 to 80 years was 24.2 months and of those over 80 years of age was 13.5 months (Figure 3.2a). Figure 3.2b-f shows the survival of the whole cohort and risk factors adversely impacting survival.

Figure 3.2: Kaplan-Meir analysis of survival of the whole cohort and risk factors adversely impacting survival. a) This shows overall survival by age groups 75-80 years and >80 year old patients with AL amyloidosis compared with those presenting at age <75 years. The median OS of the two older age cohorts is 24.2 months and 13.5 months, respectively, compared to 73 months in the younger patient cohort; b) This shows OS for patients presenting with performance status ECOG 0-1 vs. ECOG 2 vs ECOG 3-4 - median OS 45.6, 10.4 and 8.7 months respectively (log rank p <0.0001); c) This shows OS stratified by presenting dFLC <180mg/L vs ≥180mg/L - median OS 33.9 and 14.3 months respectively (log rank p =0.001); d) This shows OS by presenting NYHA class 1-2 vs. NYHA >2 - median OS 37.6 and 8.7 months respectively (log rank p<0.0001); e) This shows OS by Mayo stage I, II and III - median OS 64, 52.5 and 9.9 months respectively(log rank p<0.0001); f) This shows OS by presenting NTproBNP ≥8500ng/L vs <8500ng/L - median OS 8.7 and 30 months respectively (log rank p<0.0001).[200]

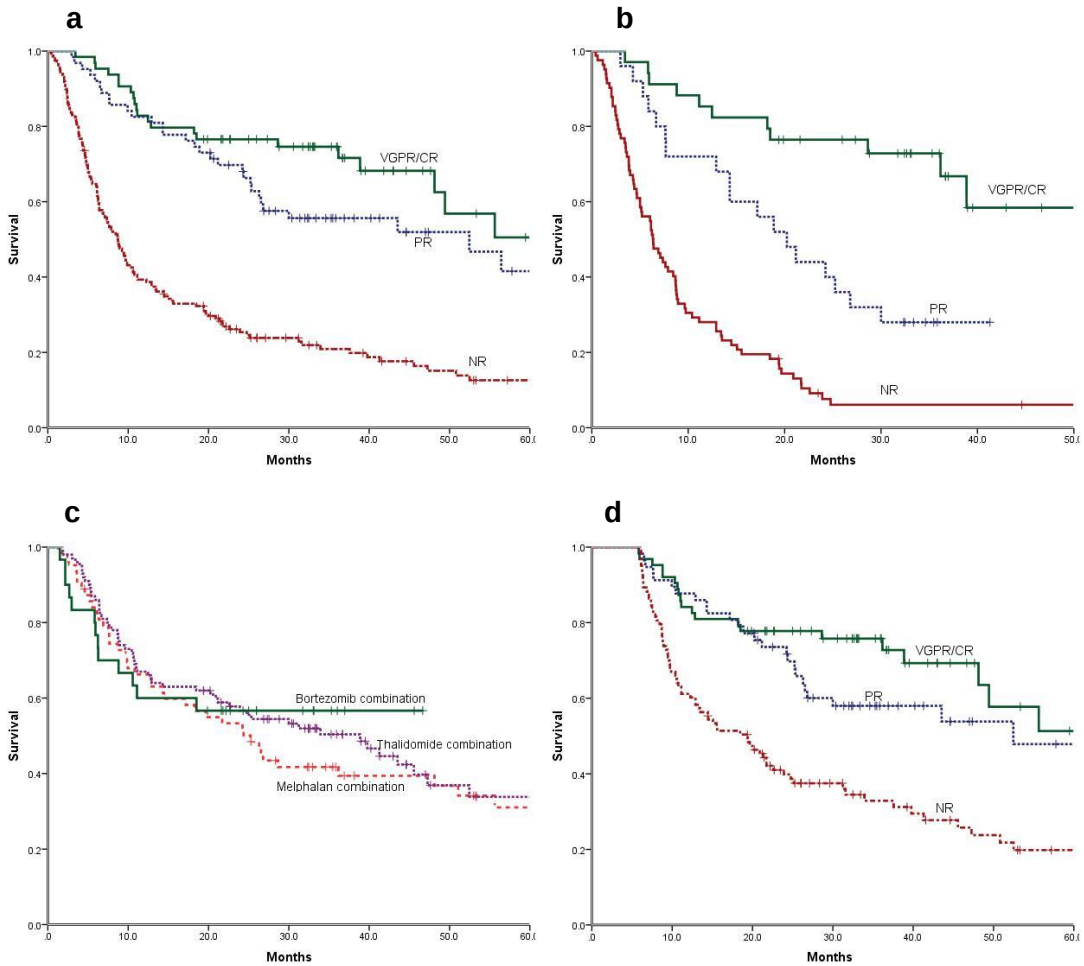

a
Survival
Months
< 75 years
75-80 years
>80 years
b
Survival
Months
ECOG: 0-1
ECOG: 2
ECOG: 3-4
c
Survival
Months
dFLC <180
dFLC >180
d
Survival
Months
NYHA 1-2
NYHA >2
e
Survival
Months
Mayo stage 1
Mayo stage 2
Mayo stage 3
f
Survival
Months
NTproBNP <8500ng/L
NTproBNP >8500ng/L

The median OS over 2005-2006, 2007-2008, 2009-2010 and 2011-2012 were 19.6, 18.5, 14.4 and 52.5 months respectively; showing an improvement in survival since 2011, perhaps due to the availability and use of novel therapeutic agents (36% of patients were treated with bortezomib based therapy during 2011-2012 period compared to only 6% in 2009-2010 and none prior to 2009) and better supportive care. Patients who received treatment had a median OS of 24.7 months compared to 8.4 months for those who chose supportive care only; $p < 0.0001$.

A CR or VGPR was associated with better median OS than a PR (74.7 months vs. 52.5 months respectively; $p = 0.037$ both on an ITT analysis and of the evaluable patients) (Figure 3.3a). The estimated five year survival in those who achieved a CR was 68% in patients aged up to 80 years and 89% in those over 80 years of age. For patients with cardiac involvement, those achieving a VGPR or CR had a median OS of 55.6 months compared to only 20.2 months for those with PR ($p=0.002$) and 6.4 months for the non-responders ($p<0.0001$) (Figure 3.3b). The survival advantage for responders was also evident within the very poor prognostic group with NT-proBNP above 8500ng/L. The responders within this group had a significantly better survival with median OS of 26.8 months compared to only 5 months in the non-responder group ($p<0.001$). The median OS of those who were treated and those who refused chemotherapy was 12.9 months and 4.8 months respectively ($p=0.009$) within this subgroup. There was an indication of better survival for patients treated with the proteasome inhibitor, bortezomib (median OS not reached) compared to melphalan (median OS 25.2 months) or thalidomide based regimens (median OS 38.9 months) (Figure 3.3c).

78% of patients who received bortezomib based regimen had achieved a haematological response with 70% achieving a VGPR or better. 65% and 67% of those on melphalan and thalidomide based regimen achieved a haematological response respectively; however, the proportion achieving VGPR/CR was slightly higher in the thalidomide (34%) group compared to melphalan group (21%). However, it is difficult to make direct comparison between the different regimens as both the reason for choice of chemotherapy and the inevitable variability in the supportive care provided could have easily influenced survival.

Figure 3.3: Kaplan-Meier analysis of overall survival based on haematological response to treatment a) Show overall survival on ITT basis by haematological response, median OS was 74.7 months in those achieving CR/VGPR and 52.5 months in those achieving a PR compared to 8.8 months in non-responders (log rank p <0.0001); b) Shows survival by response for patient with cardiac involvement in the ITT cohort – median OS 55.6 months in those with CR/VGPR and 20.2 months in those with PR and 6.4 months in NR group (log rank p<0.0001); c) Shows survival by treatment regimen – median OS not reached in bortezomib group compared to 25.2 months and 38.9 in melphalan and thalidomide groups respectively (log rank p=0.062); d) Shows OS for patients in 6 month land-mark analysis based on haematological response - median OS was 74.7 months in those achieving CR/VGPR and 52.5 months in those achieving a PR compared 19.4 months for non-responders (log rank p<0.0001).[200]



Within the ITT cohort, on univariate analysis, factors adversely impacting survival were: poor ECOG performance status, 3 or more organ involvement, cardiac involvement, advanced Mayo disease stage, high NT-proBNP levels, SBP <100mmHg, higher NYHA dyspnoea grade, peripheral neuropathy, liver involvement by ICC criteria or on SAP and dFLC ≥180mg/L. In particular, patients with NT-proBNP ≥8500ng/L (N=72) at presentation had a significantly worse outcome compared to those with NT-proBNP <8500ng/L (Figure 3.2f and Table 3.2). On multivariate analysis, independent factors adversely impacting survival were: poor ECOG status, presence of cardiac involvement (separate models run for each: cardiac involvement by either of the criteria: ICC, biomarker/echo criteria, advanced Mayo disease stage and NT-proBNP ≥8500ng/L), dFLC ≥180mg/L and achieving less than a VGPR (Table 3.2).

Table 3.2 The median survival of patients in relation to various baseline characteristics, univariate analysis and multivariate analysis at baseline, six months and at two years landmark analyses respectively.[200]

Factor	**Median survival by Kaplan Meir (months) - with and without factor**	***P* value; hazard ratio (95% confidence interval)**		
		Multivariate at baseline	**Multivariate at 6 months landmark analysis**	**Multivariate for treated patients surviving at two years**
Cardiac involvement*	45.6 vs. 12.9	**0.040**;1.47 (1.02-2.07)	**<0.0001**;2.52(1.66-3.82)	**0.030;**0.62 (0.40-0.95)
Mayo stage*	**I:** 64	Ref	Ref	Ref
	II: 52.5	0.21; 1.64 (0.76-3.54)	0.17; 1.74 (0.79-3.83)	0.17; 1.81 (0.78-4.18)
	III: 9.9	**<0.0001;** 2.0 (1.4-3.0)	**0.003;** 3.12 (1.45-6.69)	**0.006;** 3.08 (1.37-6.92)
NT-proBNP ≥8500ng/L vs. <8500ng/L*	8.7 vs. 30.0	**0.037;**1.50(1.02-2.20)		
SBP ≥100 vs. <100mmHg	25.2 vs. 6.1	**0.006**;1.86(1.19-2.90)	**0.030;** 2.03 (1.07 – 3.84)	
Liver involvement – SAP	26.8 vs. 10.6			
Liver – ICC	25.3 vs. 6.4	**<0.0001**;2.21(1.48-3.30)		**0.018;** 0.55 (0.33-0.90)
Number of organ involvement (≤2 vs. ≥3)	30.0 vs. 9.7			
ECOG performance status <2 vs. ≥2	45.6 vs. 9.7	**0.001**;1.8(1.30-2.60)		**0.012;** 0.60 (0.40-0.90)
dFLC ≥180mg/L vs. <180mg/L	33.9 vs.14.3		**0.007**;1.8(1.17-2.74)	**0.020;** 0.64 (0.44-0.93)
Haematological response: VGPR/CR vs PR			**0.014**;2.17(1.17-4.03)	**0.027;** 1.96 (1.08-3.54)
Haematological response: VGPR/CR vs NR			**<0.0001**;4.31(2.50-7.47)	**0.000;** 5.84 (3.42-9.97)

* Multivariate models were generated separately for each of the following cardiac variables: cardiac involvement, Mayo disease stage and absolute NT-proBNP < or ≥8500ng/L.

A landmark analysis was carried out for patients alive at six months. Two hundred and thirty three of the 295 patients were alive at six months. The median OS for these patients was 38.9 months. This landmark analysis confirmed that those achieving a VGPR or a CR had a superior outcome compared to those with a PR or the non-responders (median OS of patients achieving a VGPR or deeper response was 74.7 months compared to 52.5 months for lesser degrees of response, log rank $p<0.001$; Figure 3.3d). Factors adversely impacting survival in this group were similar to those identified at presentation.

One hundred and eight of the treated patients survived at least two years from diagnosis. When compared to patients who died before two years (n=113), the surviving group, unsurprisingly, had better prognostic factors. The characteristics of the patients surviving over two years are detailed in Table 3.1.

Organ responses

According to the international consensus criteria and renal response criteria, on an ITT basis of patients who received chemotherapy, 31/193(16%) had a renal response, 14/121 (12%) had a cardiac response and 5/31 (16%) had a liver response at six months. Amongst the assessable patients (i.e. excluding those who died before response assessment), 31/104 (30%) had achieved a renal response; 14/55 (25%) had a cardiac response and 5/13 (38%) had a liver response. On assessing the impact of depth of the haematological response on organ response, 58% of the renal responders,

71% of the cardiac responders and 38% of the liver responders had attained a VGPR or a CR. At 24 months, 25/193 (13%) had a renal response (of whom 50% attained a VGPR/CR), 15/121 (12%) had a cardiac response (of whom 73% attained a VGPR/CR) and 5/31 (16%) achieved a liver response (of whom 60% attained a VGPR/CR).

Discussion

Improved awareness of AL amyloidosis and the possibilities for its treatment allows for the prospect of more frequent diagnosis of AL amyloidosis in all age groups. In particular, the general longevity and availability of non-invasive investigative modalities suggest that AL will be increasingly recognized in older individuals especially as the prevalence of MGUS, a usual precursor of AL, rises with age. There are no studies, to the best of our knowledge, focusing specifically on AL amyloidosis among older patients. Hence little is known about its true natural history or its potential to respond (or not) to chemotherapy. Chemotherapy in AL amyloidosis is challenging in patients of all age groups due to multisystem vital organ dysfunction, reducing its tolerability and increasing the likelihood of treatment related toxicities. These challenges have historically led to many older patients being denied therapy. In general, amongst cancer patients, increasing age and co-morbidities are associated with reduced use of all therapies including surgery, chemotherapy, and radiotherapy; consequently, otherwise healthy cancer patients frequently do not receive appropriate treatments.[201] A UK Department of Health document suggested that

clinicians may place too much emphasis on chronological age as a proxy for other factors which are often but are not necessarily associated with age, such as co-morbidities and frailty.[202] There are ongoing efforts in the myeloma community to define frailty in order to obtain uniformity in clinical trials and develop guidance for treating physicians on dose modification of currently used treatments. The issues of frailty due to co-morbidities that are likely to worsen with treatment, and frailty caused by the illness for which treatment is being given, and which may be partly reversible, remains a difficult area to navigate.

This study was specifically designed to assess the clinical features and course of AL amyloidosis in elderly patients. The median age at diagnosis of patients with AL amyloidosis is ~ 60 years[29, 195] and nearly a fifth of all patients seen at the UK National Amyloidosis Centre were aged >75 years. The presenting features of patients aged >75 years were, in general, similar to younger AL patients with a mild male predominance and similar patterns of organ involvement. The majority of patients had renal involvement, followed by cardiac and liver amyloidosis. Fifty four percent of all patients had Mayo stage III disease at presentation compared to ~40% in younger patients[142] raising a serious concern that there may be a greater delay in diagnosis of amyloidosis in this elderly patient cohort where symptoms may well have been attributed to other co-morbidities. Although, in the UK, a substantial majority of patients with amyloidosis are seen at our national referral centre, we acknowledge that patients who are very elderly with poor performance status may either not be referred or are too unfit to travel – a possible bias in this study. Cardiac amyloidosis in the elderly is an

area of increasing interest not only in relation to AL type, but also because of wild type transthyretin (ATTRwt) which is increasingly being recognized as the cause of heart failure with preserved ejection function in older patients.[203] Therefore, it is important to acknowledge that cardiac involvement may have been over estimated in this aged population due to multiple factors, the potential overlap of ATTRwt amyloid coexisting with the AL and/or hypertensive heart disease. Use of non-invasive diagnostic tests for cardiac amyloidosis is important given the well-recognized limitations of cardiac biomarkers in older individuals.[204] Modalities such as cardiac magnetic resonance imaging[205], which has a much higher sensitivity and specificity for cardiac amyloidosis than echocardiography may assist differentiation of AL and ATTRwt amyloidosis. In addition, due to the availability of treatment options for the ATTRwt, early use of non-invasive radionuclide imaging with MRI, ^{99m}Tc-DPD[206] or ^{18}F-florbetapir[207] should be considered to avoid diagnostic delay.

The decisions to proceed with treatment and the type of chemotherapy in elderly patients may be influenced by factors including social situations that may not be an issue in younger individuals. One fifth of patients in our cohort made an informed decision not to receive chemotherapy and chose supportive care only. This was a frail group, with 39% aged over 80 years, with poor ECOG status and advanced cardiac involvement. Perhaps due to the limitations of cardiac biomarkers in the older patients, biomarker based staging was not always helpful in identifying the patients with poorest prognoses – a fifth of Mayo stage III patients survived for more than 2 years. By contrast, functional markers such as

NYHA and ECOG, which reflect patients' overall physiological state, appeared to have better discriminatory capacity, since only 10% of those with either NYHA 3-4 or ECOG ≥3 were alive at 2 years and had a median OS of 9 months.

The chemotherapy regimens used in the study reflected those in practice generally in the UK during the study period. Thalidomide based combinations were commonest in the last decade, with a recent move toward bortezomib based regimens. Treatment was evidently challenging with only a third of all patients completing the planned six cycles of chemotherapy and three quarters of patients reporting major toxicity. Thalidomide based treatments appeared to be particularly poorly tolerated in this patient group. There was a suggestion of better tolerance and higher responses with bortezomib based regimens in this cohort but further studies are needed before any firm conclusions can be reached.

The overall hematologic response rate of only 44% based on this standard ITT analysis of the whole cohort may appear disappointingly low, but when the analysis is performed excluding patients who opted not be treated, 63% achieved a clonal response including a third achieving VGPR or better. This response rate compares well to responses reported by our group and others using chemotherapy combinations with AL amyloidosis in general.[135, 142] Whereas, the median OS of the whole cohort of just over two years (24.2 months and 13.3 months in the 75-80 and >80 year groups respectively) is inferior to the 3-4 year survival of the AL population in general [196], deeper clonal responses translated into an excellent survival advantage (the 5 year survival amongst haematological responders was 45%

with a median OS 6.2 years for those achieving a VGPR or better). Although, numbers were small, the estimated 5 year survival for an elderly patient achieving a CR was 76% in concordance with AL amyloidosis in general.[120, 208] When compared to the UK population in general, the Office for National Statistics in England and Wales has projected the life expectancy for a 75 year old male and female to be 11 and 13 years, and that for an 80 year old male and female as 8.2 and 9.6 years respectively.[209] With the outcome of patients in CR approaching this figure, our data supports treating older patients with high efficacy regimens aiming to achieve deep clonal responses.

Cardiac involvement is the most important determinant of clinical outcome in patients with AL amyloidosis[71] in general, and was also associated with poor outcome in this cohort of older patients.[71] Other factors adversely impacting outcomes at presentation on univariate analysis were similar to those for AL in general including advanced Mayo stage disease, NTproBNP >332ng/L, systolic SBP <100mmHg, dFLC ≥180mg/L, liver involvement, ECOG performance status of ≥2 and 3 or more organ involvement. On multivariate analysis, independent factors adversely impacting survival were NTproBNP >332ng/L or advanced Mayo stage or cardiac involvement (independently analysed), liver involvement by ICC, SBP <100mmgHg, dFLC ≥180mg/L and ECOG ≥2. These factors remained significant on the landmark analysis of the 233 (79%) patients surviving 6 months. On both the 6 month and two year landmark analyses, additional factor which independently impacted survival was achieving a haematological response to treatment. In AL amyloidosis, the final aim is for

the haematological responses to eventually translate into organ responses, but the latter are often much delayed and organ function may continue to improve for a long period in association with a sustained clonal response. We attempted to define the characteristics of older patients who survive long enough to potentially benefit from the treatment in terms of organ response. We chose two years as the time point which was the median of our series although recent data on cardiac and renal responses suggests that earlier time points may also be useful. At 2 years, on an ITT basis of patients who received treatment, 13% achieved a renal response, 12% had a cardiac response and 16% had a liver response. A high proportion of organ responders had achieved a ≥VGPR to chemotherapy. This gratifyingly confirms that, striving for an excellent haematological response is crucial since such responses translate into a high proportion of organ responses even in elderly patients. The patients who survived more than two years had less number of organs involved, particularly less cardiac involvement (40% vs. 70%), lower dFLC (113mg/L vs 224mg/L), higher presenting SBP (only 4% with SPB <100mm), markedly lower NT-proBNP (843ng/L vs. 4093ng/L) and lower troponin-T. Strikingly, 95% and 72% of the 2 year survivors had a presenting NYHA 1-2 and ECOG 0-1 respectively.

Younger patients with AL amyloidosis may be salvaged with second line treatment if response to first line treatment is poor, but the decline in performance status among elderly patients due to first line treatment toxicity and disease progression may preclude further therapy. More knowledge is required to enable refined patient selection, with the dual objectives of avoiding toxicity from unhelpful treatment whilst permitting treated patients to

have the best chance of achieving a deep clonal response; thus, age alone should not be used as a surrogate of fitness for treatment. Critical questions about the choice of initial therapy, the actual schedule of the regimen, especially steroid doses and dose modifications remain unanswered by this retrospective analysis. Similarly, toxicity data remains limited. Our current prospective ALCHemy study may answer some of these questions.

In summary, the presentation of elderly patients with systemic AL amyloidosis is similar to that of AL population in general but there are a higher proportion of patients with advanced stage disease, perhaps reflecting delay in diagnosis. Outcomes of responders to treatment are good, especially so in those achieving a VGPR or better which translate into organ responses. However, treatment toxicity impedes on the tolerability and consequently the possibility of achieving a deeper haematological response. Choosing an appropriate highly effective first line treatment appears crucial as patients may not remain fit for salvage therapies. Excluding the very frail patients with advanced organ involvement who require careful counselling about risks vs. benefits of treatment, this study strongly supports the use of rapidly effective frontline treatment for older patients with systemic AL amyloidosis, striving for an early deep clonal response with good prospects of long term survival. Prospective studies in older patients with novel agents with a better toxicity profile and ease of administration, such as oral proteasome inhibitors, may allow a greater proportion of this subgroup of patients to benefit from treatment.

Chapter Four: Immunoglobulin D-associated AL amyloidosis – the clinical profile and treatment outcomes

This chapter is written in the context of my publication: Clinical profile and treatment outcomes of immunoglobulin D associated AL amyloidosis. Roussel M, Sachchithanantham S, Gibbs SD, Venner CP, Pinney JH, Gillmore JD, Lachmann HJ, Hawkins PN, Wechalekar AD. Br J Haematol. 2013 Sep;162(6):856-8. Copyright permission obtained from Oxford University Press, licence no. 4037321063107 for use in my thesis

Introduction

Chapter four focuses on the very rare subtype, IgD-related AL amyloidosis.

IgD-monoclonal gammopathies are an uncommon phenomenon, accounting for less than five percent of patients with myeloma. IgD-monoclonal gammopathy of uncertain significance (MGUS) is exceptionally rare with less than a dozen cases reported in the literature.[27] Given that most patients with AL amyloidosis have an underlying MGUS, remarkably few patients with AL amyloidosis consequent to serum IgD-paraprotein have been reported. IgD-related amyloidosis may be misdiagnosed as light chain amyloidosis if patients with a serum light chain band are not routinely

screened for the presence of an IgD-paraprotein. Only 53 such patients were identified among the extensive experience of AL amyloidosis at the Mayo Clinic over a 41-year period.[210] This subgroup supposedly had a distinct phenotype – exhibiting lower incidence of cardiac and renal involvement with overall survival not significantly different from the other AL amyloidosis patients. This was surprising since IgD multiple myeloma is known to have a much worse prognosis and a greater degree of renal insufficiency at presentation.

In this chapter, the experience over a 12 year period at the UK NAC is explored, describing the clinical profile of IgD-related AL amyloidosis and also the treatment outcomes in this subgroup of patients is presented.

Methods

Patient selection

Among 2861 patients with AL amyloidosis seen between 2000 and 2012, serum IgD-monoclonal protein was identified in 20 (0.7 %) patients, who were included in this retrospective study. At the NAC, all patients are routinely screened for the presence of IgD-monoclonal protein. IgD-related AL amyloidosis was defined as all patients with confirmed AL amyloidosis with demonstrable IgD-paraprotein.

Outcome measures and Statistics

Outcome measures comprised of overall patient survival (OS) and hematologic response to first line treatment. The primary outcome measure was OS. Statistical analysis was undertaken using the SPSS 21 software package (SPSS, Chicago, IL). Survival was assessed by the method of Kaplan and Meier and compared by log-rank test. All *P* values were 2 sided with a significance level of 0.05.

Results

Patients, disease characteristics and laboratory findings

Fifty-five percent of the patients with IgD-AL amyloidosis were male and the overall median age was 64 years (range 51-84). All patients had a detectable IgD band on IFE. Eight patients had measurable IgD-monoclonal band on serum electrophoresis with a median of 1.5g/L (range 1.0 - 3.5g/L). All patients except one had evaluable FLC with abnormal FLC ratios. Eighteen (90%) were IgD lambda and 2 (10%) were IgD kappa on serum immunofixation. Median serum involved free lambda and kappa light chains levels were 540mg/L (range 53-6000mg/L) and 387mg/L (range 122-651), respectively. Nine (45%) patients had an underlying (asymptomatic) myeloma with over 10% plasma cells in bone marrow and two had symptomatic myeloma. As per the International Amyloidosis Consensus Criteria (ICC)[69], kidneys were the commonest organ involved in 15 patients (75%) with 30% presenting with creatinine clearance less than 50 ml/min and

a median 24-hour proteinuria of 2.8g (range 0.5-14.8g). The median serum albumin levels were 33g/L (range 19-47mg/L). Fifteen (75%) patients had cardiac involvement by ICC. Eight patients had Mayo stage II and four patients had Mayo stage III cardiac biomarkers with median NT-proBNP of 376pMol/L (range 13-3558 pMol/L) and cardiac troponin T of 0.03µg/L (range 0.01-0.19µg/L) for the whole cohort. Four (20%) patients had neuropathic disease of whom one had peripheral and three had autonomic neuropathy. Four patients showed liver uptake on ^{123}I labelled SAP scintigraphy but none had liver involvement according to the ICC (median alkaline phosphatase levels 110UI/L, range 44-203UI/L).

Treatment

The first line treatment regimens were as follows: seven patients received cyclophosphamide-thalidomide-dexamethasone (CTD), four had vincristine-adriamycin-dexamethasone (VAD), one had cyclophosphamide-bortezomib-dexamethasone (CVD), one had upfront autologous stem cell transplantation (ASCT) and three were treated with oral or intravenous melphalan. Of note, two patients received consolidation ASCT (one following CTD and the other after VAD induction). One patient refused treatment and three died before receiving any chemotherapy.

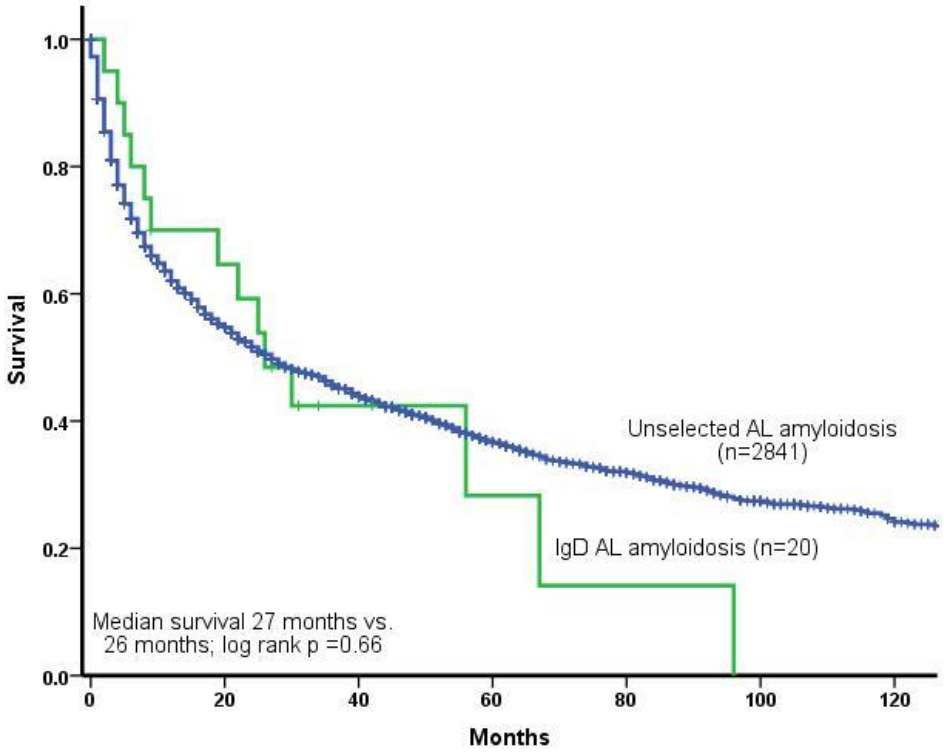
Overall haematological response rates on an intention to treat basis was 50% and was 62% in the 16 patients who actually received treatment with 4 (25%) and 6 (37%) of evaluable patients achieving complete (CR) and partial (PR) responses, respectively. The overall dFLC response rate was

63% including dFLC-very good partial response (defined as a greater than 90% reduction of dFLC from baseline) or better in 7 (43%) patients. The haematological responses in IgD amyloidosis to front line therapy appear similar to the responses noted for non-IgD patients in other studies.[196] Six (32%) patients needed further therapies for progressive disease; this included one patient who relapsed with myelomatous bony lesions. Three of these patients received a bortezomib-based second line therapy and the remaining three received a thalidomide based regimen.

Clinical outcome

Median follow-up from diagnosis was 22 months (range 2-93 months). Fourteen of the 20 (70%) patients have died; seven (35%) of which were within 12 months of diagnosis. Of note, 3 patients died at 30, 56 and 67 months from diagnosis, respectively, two were due to progression of multiple myeloma which is unusual in AL amyloidosis and the third due to prostatic cancer. The estimated median overall survival (OS) was 27 months (Figure 4.1). Although this was not significantly different when compared to the non-IgD patients, the estimated 5-year OS was 26% for IgD patients compared to 37% for non-IgD patients over the same time period.

Figure 4.1 Overall survival of patients with IgD-associated AL amyloidosis compared to all non-IgD patients seen in a 12-year period showing no significant difference in the median survival but with a suggestion of poorer outcomes of IgD patients in the longer term.



Discussion

In summary, IgD associated AL amyloidosis is rare and is predominantly of the lambda subtype. The clinical phenotype mirrors AL amyloidosis in general which could be explained by the fact that the amyloidogenic light chains, and not the intact M-protein, lead to the amyloid phenotype. Compared to a previously reported series from the Mayo clinic, interestingly, the patients in this study had a much higher proportion of renal and cardiac involvement at presentation, which is similar to non-IgD associated AL amyloidosis. The early outcomes of IgD-AL amyloidosis appear to be similar to previously reported outcomes with AL amyloidosis in general.[211] In addition, the treatment responses in this subgroup seem to be similar to that of non-IgD AL amyloidosis.

The early prognosis of amyloidosis is driven by the amyloidotic organ involvement rather than by the underlying plasma cell dyscrasia – however, the longer term prognosis may depend on the sensitivity of the plasma cell clone to therapy. Patients with IgD-AL amyloidosis present with higher clonal burdens than AL in general. Although the overall survival of the patients in this series is similar to AL in general, there is a suggestion of inferior 5 year survival in the IgD cohort perhaps due to factors such as the higher plasma cell burden and clonal resistance playing a vital role in the overall outcome. Interestingly, two patients progressed to symptomatic myeloma which is uncommon in AL amyloidosis; perhaps, suggesting that unusually for amyloidosis, IgD-AL patients maybe at a risk of progression to symptomatic myeloma.

In summary, IgD-associated amyloidosis is a rare disease but has a common AL phenotype. The response to initial treatment and median overall survival are similar to AL in general although there is a suggestion of inferior five year survival. Patients present with higher clonal burdens than AL in general and there appears to be higher risk of progression to symptomatic myeloma.

Chapter Five: Immunoglobulin M-related AL amyloidosis - the natural history, outcomes and validation of existing prognostic/response criteria.

This chapter is written in the context of my publication: European Collaborative Study Defining Clinical Profile Outcomes and Novel Prognostic Criteria in Monoclonal Immunoglobulin M-Related Light Chain Amyloidosis. Sachchithanantham S, Roussel M, Palladini G, Klersy C, Mahmood S, Venner CP, Gibbs S, Gillmore J, Lachmann H, Hawkins PN, Jaccard A, Merlini G, Wechalekar AD. J Clin Oncol. 2016 Jun 10;34(17):2037-45. Copyright permission obtained from Oxford University Press, licence no. 4036751396108 for use in my thesis

Introduction

Chapter four explored the clinical features and outcomes of one of the rare subgroups, IgD-related AL amyloidosis. Chapter five will explore another rare subgroup, IgM-related amyloidosis.

An intact monoclonal immunoglobulin protein (M-protein) can be identified in about 45-55% of patient with AL amyloidosis. The M-protein is usually IgG or IgA paraprotein associated with an underlying plasma cell disorder. However, in 5-7% of patients, AL amyloidosis is associated with an underlying IgM paraprotein, described in small series by several groups.[27,

[212, 213] It has previously been suggested that IgM-AL amyloidosis should be classed as a distinct clinical entity with several distinguishing clinical features from that of non-IgM AL amyloidosis.[214, 215] Given its rarity, IgM-AL remains poorly studied. Since this disorder is different, as all patients have an intact monoclonal protein and appear to have an underlying lymphoproliferative disorder, criteria validated for non-IgM AL have not been formally tested in this disease. The treatment paradigms designed for non-IgM AL have been used in IgM-AL amyloidosis, which may not always be appropriate.

Chapter five reports the clinical characteristics, and outcomes in a large series of 250 patients with IgM-associated AL amyloidosis seen at three major European amyloidosis centres. This chapter also seeks to analyse the utility of prognostic and response criteria, validated in non-IgM AL amyloidosis in this rare and distinct sub-group of patients. This is the largest series on IgM-related AL amyloidosis.

Methods

Patient selection

Two hundred and sixty one newly diagnosed patients with IgM-associated AL amyloidosis from amyloidosis centres in London (United Kingdom, 149 patients), Pavia (Italy, 81 patients) and Limoges, (France, 31 patients) between January 1990 and December 2012 were, retrospectively, included in this study.

Outcome measures and Statistics

Outcome measures comprised of overall patient survival (OS), hematologic response (HR) to first line treatment and organ response. The primary outcome measure was OS. The validity of currently published staging and response criteria in non-IgM AL were applied to this series to assess the utility of those criteria in this patient cohort including the impact of HR on the survival of this group of patients. HR were assessed as per the consensus criteria published by Palladini *et al* [130] and by use of serum paraprotein (PP) response.

Survival was described by its median and presented graphically by Kaplan-Meier curves. The association of a series of candidate predictors and survival were assessed by Cox models. The proportional hazard assumption was tested and satisfied in all cases. Linearity of ordinal predictors was verified by the likelihood ratio test to compare nested models. Response was treated as a time dependent variable. The effect modification on the relationship of response and survival by Mayo Stage was assessed by including an interaction term in the model. All non-co-linear variables with p-value <0.1 at univariate analysis and with missing data below 20% were included in a multivariable Cox (time-dependent) regression model. For all Cox models, clustered robust standard errors were computed to account for within-country correlation. Model validation was performed by calculating the shrinkage coefficient/noise for calibration and the Harrell's c statistic for discrimination. A 2-sided p-value <0.05 was considered statistically significant.

Results

Two hundred and sixty one patients with isolated IgM paraprotein associated AL amyloidosis were identified from three European centres. Eleven patients had localised amyloidosis and were, therefore, excluded from analysis. Two hundred and fifty (95%) patients had systemic AL amyloidosis and were included in this retrospective study. The baseline demographics are given on Table 5.1. Forty five percent of those referred before 2004 were over 67 years of age, this increased in 2004-2009 period to 51% and then to 64% in 2010-2012. Cardiac, renal, soft tissue and liver involvement were seen in 45%, 68%, 35% and 17 % of patients at diagnosis. 40%, 34% and 26% of patients had Mayo stage (data available in 216 (86%) patients) I, II and III disease respectively. Lymph node involvement was detected in 20% of patients at presentation.

Table 5.1 Patient demographics at presentation[216]

	Median	No of patients (%)	Missing data (%)
Age at presentation	67 (38-89)	250	0
Sex (Male: Female ratio)	1.7:1		0
Paraprotein concentration (g/L)	10 (IF-70)		35 (14)
Monoclonal light chain type			0
• Kappa		100 (40)	
• Lambda		150 (60)	
Abnormal FLC ratio		163 / 221 (74)	30 (12)
Evaluable FLC		147 / 221 (67)	30 (12)
dFLC (mg/l) at presentation • Kappa • Lambda	122.3 (30-7762) 100.5 (30-1343) 155 (41-7762)		
Hemoglobin (g/L)	12.5 (7.8-17.7		32 (13)
Total white cell count (x 10^9/L)	7.04 (0.56-23)		75 (30)
Platelets (x 10^9/L)	294.5 (18-757)		75 (30)
Creatinine (µmol/L)	97.2 (42-ESRD)		5 (2)
Albumin (g/L)	35 (12–49)		28 (11)
Alkaline phosphatase (IU/L)	129 (42-3488)		32 (13)
24 hour proteinuria (g/24 hrs)	1.78 (0-45)		20 (8)
Creatinine clearance (ml/min)	64 (ESRD-157)		137 (55)
Organ involvement			3 (1)
No of organs involved	2 (1-6)		
• 1		81 (32)	
• 2		89 (36)	
• 3 or more		80 (32)	
Cardiac		112 (45)	2 (0.8)
• NT-proBNP (ng/L) • NT-proBNP >8500 ng/L	609 (17-120737) 19 (9%)		35 (14)
• cTnT (ng/ml) • cTnI (ng/ml)	0.020(0.003-0.467) 0.020(0.002-0.599)		55 (22)
• IVS (mm)	12 (7-22)	232 (79)	50 (20)
Mayo stage		216 (86)	35 (14)
• Stage I		87 (40)	
• Stage II		73 (34)	
• Stage III		56 (26)	
Renal		169 (68)	0
Liver		41 (17)	0
Soft tissue • Lymph node		80 (35) 50 (20)	0
PNS		37 (15)	0
ANS		32 (13)	1 (0.4)
GI		22 (9)	3 (1)

A total of 131 (52%) patients had a clearly identifiable lymphoproliferative disorder (predated the AL diagnosis in 39). Thirty four (14%) had a normal BM biopsy with no detectable clonal dyscrasia. Fifteen (6%) had excess plasma cells in the BM. Details of BM were not available for 70 patients (28%). Of the patients with an underlying lymphoproliferative disorder, 97 (39%) had lymphoplasmacytic lymphoma and 34 (14%) had a Non-Hodgkin's lymphoma (NHL) not specifically classified. Two patients had chronic lymphocytic lymphoma and further two patients had Follicular Lymphoma.

Treatment and response

Two hundred and twenty eight (91%) patients received treatment and eight died prior to starting chemotherapy. Fourteen patients were excluded from treatment analysis as information on treatment was not available. Twenty two different combination of regimen were used as first line therapies. These were grouped into ten categories for ease of analysis and are shown on table 5.2.

Table 5.2 Haematological response, median OS, two year survival and time to next treatment (TTNT) for each treatment group[216]

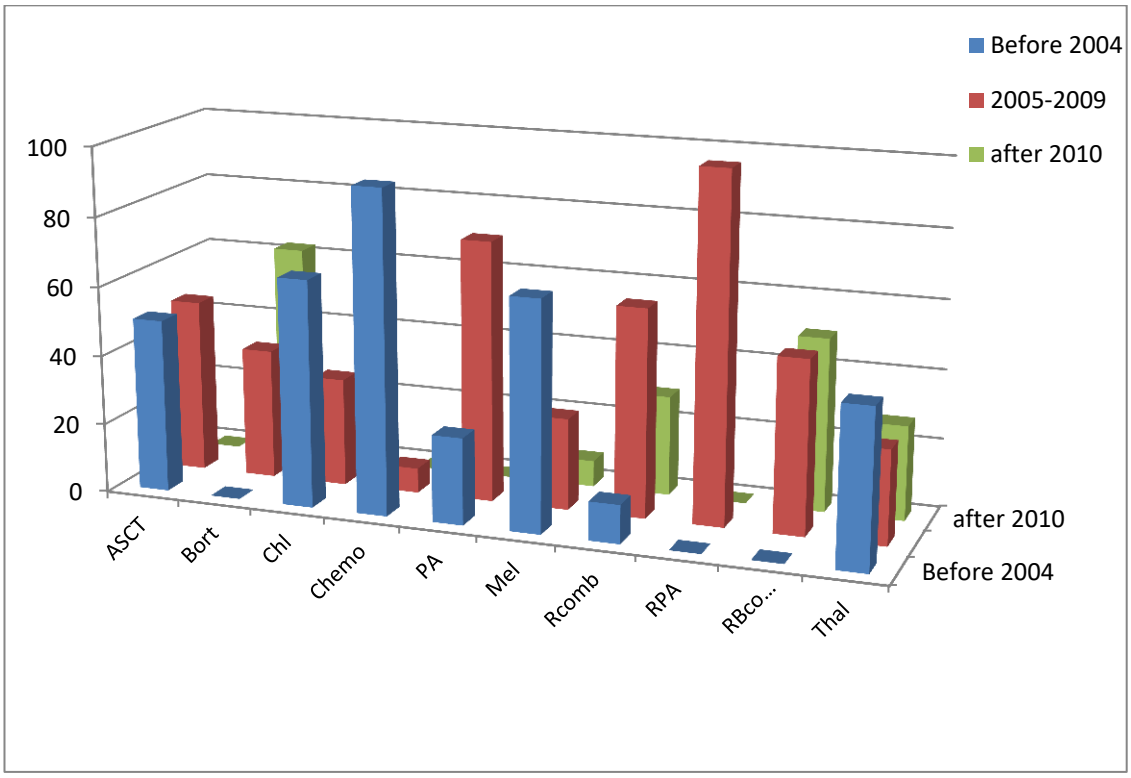
Treatment type	N (%)	Proportion with cardiac involvement (Mayo stage III, %)	PR or better % (VGPR or better, %)	Median OS (Months)	2 year survival (%)	TTNT (Months)
ASCT	4 (1.8)	25 (0)	100 (33)	NR	100	NR
Chlorambucil / Cyclophosphamide	62 (27.1)	41 (25)	46 (7)	50.8	73	11
CHOP/COP/VAD	14 (6.1)	21 (33)	62 (0)	49.8	79	21
Melphalan +/-Dex	53 (23)	58 (28)	70 (26)	22.9	49	8
FC/CLAD	12 (5)	42 (25)	40 (0)	31.4	58	10
FCR	11 (4.8)	27 (0)	70 (30)	69.4	73	63
RCD/RCHL/RCVP/RCHOP/RTD	45 (19.7)	44 (23)	63 (15)	91.9	63	20
Bortezomib	8 (3.5)	50 (25)	57 (42)	NR	88	NR
Rituximab+Bortezomib	8 (3.5)	50 (25)	86 (29)	30.2	75	19
Thalidomide	11 (4.8)	36 (27)	63 (9)	37.9	55	5

NR – Not reached.

The median number of lines of therapies was one with a range of 1-5. Figure 5.1 shows the changing trend in treatment profile since the year 1990. The use of conventional chemotherapy, chlorambucil and melphalan, has diminished over time. Purine analogues, traditional chemotherapy regimens and thalidomide were predominantly used between 2005 and 2009. Since 2010, the monoclonal antibody, rituximab, was most frequently used in

combination with bortezomib or combination chemotherapy (R-CD or R-CVP/CHOP).

Figure 5.1 Shows the change in treatment trend over time for the ten different treatment groups[216]; ASCT – Autologous Stem Cell Transplantation, Bortezomib based regimens, Chlorambucil, Conventional chemotherapy - CHOP/COP/VAD, PA – Purine Analogues, Melphalan, Rituximab + Conventional chemotherapy, RPA – Rituximab + Purine Analogues, RBortezomib – Rituximab + Bortezomib and Thalidomide based regimens, for the time period – pre 2004, 2005-2009 and after 2010.



Two hundred and twelve of the treated patients had evaluable paraprotein (81 by paraprotein alone) or dFLC (12 by dFLC alone) and 119 by both. HR data was available for 172 patients (78%) (M-protein data in 49 patients as dFLC not evaluable). On an ITT analysis, 102 (57%) patients achieved HR (43% partial response (PR), 9% very good partial response (VGPR) and 5% complete response (CR)). Of the 49 patients evaluable for M-protein only response, 24 achieved PR, one CR and 24 were non-responders. Fifteen patients deemed as non-responders on the basis of M-protein alone, had achieved PR (13) and VGPR (2) by dFLC response. Table 5.2 details treatment regimens, HR with proportion achieving VGPR or better, median OS as well as two year survival rates and time to next treatment for patients treated with the various first line therapies. The overall responses appeared best with ASCT, R-bortezomib, followed by FCR/R-Cladribine and Melphalan-Dexamethasone. However, the numbers are too small in individual treatment groups for meaningful statistical comparisons.

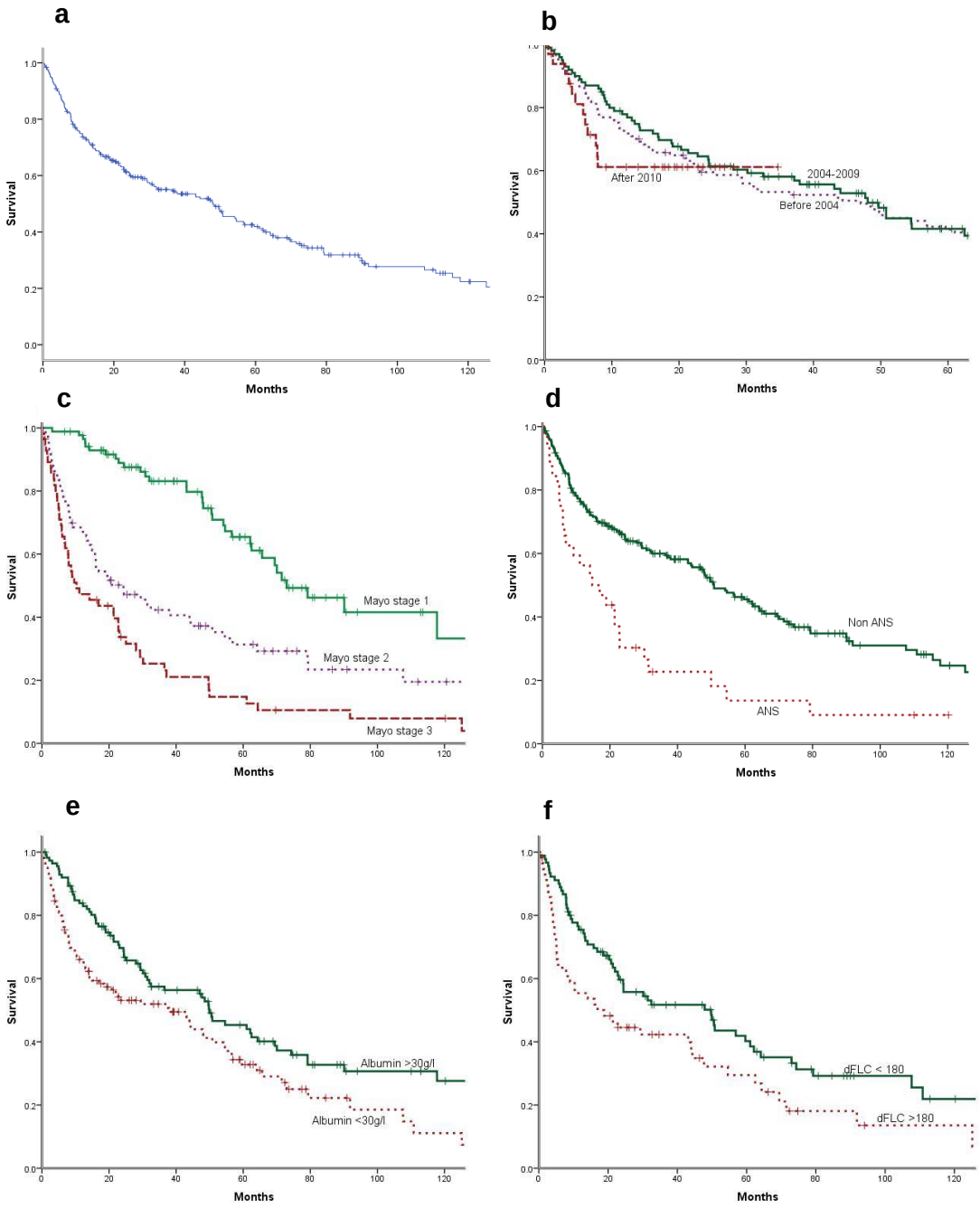
Survival analysis

The median overall survival of IgM-related systemic AL amyloidosis patients was 47.9 months (figure 5.2a). There was no improvement in survival over the study period, as shown in figure 5.2b: The best outcome was seen in patients with no identifiable clonal infiltrate in the BM (54 months) when compared to those with a lymphoid infiltrate or a plasma cell predominant infiltrate (44 months and 23 months respectively). Patients under the age of 67 years (median age), had a significantly better survival rate compared to

those over 67 years at presentation (62 months vs. 29 months respectively, p<0.001).

Survival by disease characteristics are shown on Figure 5.2c-f. The presence of cardiac involvement conferred significantly worse outcomes (median OS 21 vs. 62.5 months for no cardiac involvement), as did advance Mayo disease stage (median 73, 24 and 10 months for stage I, II and III respectively). Other factors associated with poorer outcomes were, peripheral neuropathy (PN) or autonomic neuropathy (AN), low serum albumin (<30g/L) (29 vs 50 months, p=0.008), higher dFLC (>180 mg/L) (18.9 vs 48 months, p=0.021) and liver involvement.

Figure 5.2 Shows survival curves[216]: **a)** Overall survival of patients with IgM-related AL amyloidosis with median survival of 47.9 months; **b)** Survival over time - there was no improvement in the survival over the study period. Median OS - 48 months before 2004, 50 months for 2005-2009 and not reached for 2010 -2012; **Figures c-f** show survival by organ involvement: **c)** Survival curves by Mayo stage - median OS for stage I, 73 months, stage II, 24 months and stage III, 10 months (log rank p <0.001); **d)** Autonomic nervous system (ANS) involvement vs no involvement, median OS 15 months and 51 months respectively (p<0.001); **e)** albumin <30g/l vs >30g/l, median OS 29 months and 50 months respectively (p=0.008); **f)** dFLC >180mg/L vs dFLC <180mg/L, median OS 19 months and 48 months respectively (p=0.021).


a
Survival
Months
b
After 2010
2004-2009
Before 2004
Survival
Months
c
Mayo stage 1
Mayo stage 2
Mayo stage 3
Survival
Months
d
Non ANS
ANS
Survival
Months
e
Albumin >30g/l
Albumin <30g/l
Survival
Months
f
dFLC < 180
dFLC >180
Survival
Months

In this cohort, only 13% of patients with neuropathy received bortezomib or thalidomide. Table 5.3 details univariate and multivariate analysis of factors affecting the overall survival. Due to co-linearity of cardiac variables, different multivariate models of NT-proBNP and Troponin are also given in table 5.4.

Table 5.3 Factors affecting overall survival – univariate and multivariate analysis[216]

Factor	**Median survival (months)**	**Univariate HR (95%CI); p-values**	**Multivariate HR (95%CI); p-values Noise in model: 0.10 Harrell's C coef: 0.76**	**Multivariate HR (95%CI); p-values Noise in model: 0.07 Harrell's C coef: 0.78**
Age (years) (<67 vs >67)	62 vs 29	1.64 (1.40-1.92); <0.001	1.96(1.64-2.35); <0.001	1.89(1.59-2.24);<0.001
Paraprotein >10 vs <10	48 vs 50	1.27 (1.04-1.54); 0.019	1.33 (0.89-2); 0.165	1.34(0.88-2.06);0.174
dFLC (mg/l) (<180 vs >180)	48 vs 19	1.51 (1.07-2.15); 0.021		
NHL type				
MGUS	54	Ref		
WM/LPL	38	1.43 (0.67-3.06); 1.000		
Other NHL	50	1.35 (0.62-2.94); 1.000		
PC	23	1.54 (0.94-2.54); 0.131		
Cardiac vs Non Cardiac	21 vs 62	2.34 (1.65-3.30); <0.001		
Mayo stage			p<0.001	P<0.001
Mayo stage I	73	Ref	1	1
Mayo stage II	24	2.63 (2.14-3.24); <0.001	2.33(2.27-2.39); <0.001	2.31(2.15-2.49);<0.001
Mayo stage III	10	4.46 (3.11-6.39); <0.001	4.24(2.94-6.11);<0.001	4.1(2.52-6.68);<0.001
Nt-proBNP(ng/l) (>332 vs <332)	19 vs 73	3.15 (2.66-3.72); <0.001	Not included	Not included
cTnT >0.035 µg/L or cTnI>0.1µg/L	10 vs 57	2.79 (1.96-3.97); <0.001	Not included	Not included
Soft tissue vs no Soft tissue	44 vs 55	0.77 (0.49-1.20); 0.244	1.41(0.81-2.46);0.222	1.38(0.77-2.47);0.281
PNS vs no PNS	23 vs 50	1.54(1.21-1.95); <0.001	2(1.9-2.12);<0.001	1.98(1.79-2.19);<0.001
ANS vs no ANS	15 vs 51	2.27 (1.53-3.37); <0.001	2.04(1.77-2.36);<0.001	2.17(1.68-2.81);<0.001
GI vs no GI involvement	24 vs 49	1.19 (0.78-1.84); 0.420	Not included	Not included
Renal vs non Renal	43 vs 55	1.26 (0.91-1.75);	0.86(0.62-	0.85(0.64-

		0.171	1.19);0.361	1.15);0.295
Liver vs non Liver	21 vs 51	1.36 (1.22-1.52); <0.001	1.32(1.09-1.59);0.004	1.36(1.07-1.72);0.011
Albumin (≥30g/l vs <30g/l)	50 vs 29	0.64 (0.46-0.89); 0.008	0.56(0.25-1.22);0.145	0.55(0.25-1.21);0.138
Organ involvement				
1	69	Ref		
2	48	1.34 (0.79-2.29); 0.563		
≥3	19	2.42 (1.73-3.37); <0.001		
Haematological response vs no response	69 vs 28	0.58 (0.38-0.88); 0.012	Not included	0.66(0.38-1.15);0.141
Type of haematological response				
NR	28	Ref		
PR	64	0.64 (0.40-1.04); 0.073		
CR/VGPR	Not reached	0.36 (0.21-0.61); <0.001		

NR – Non responders

Table 5.4 Showing NT-proBNP and Troponin in two separate multivariate analyses[216]

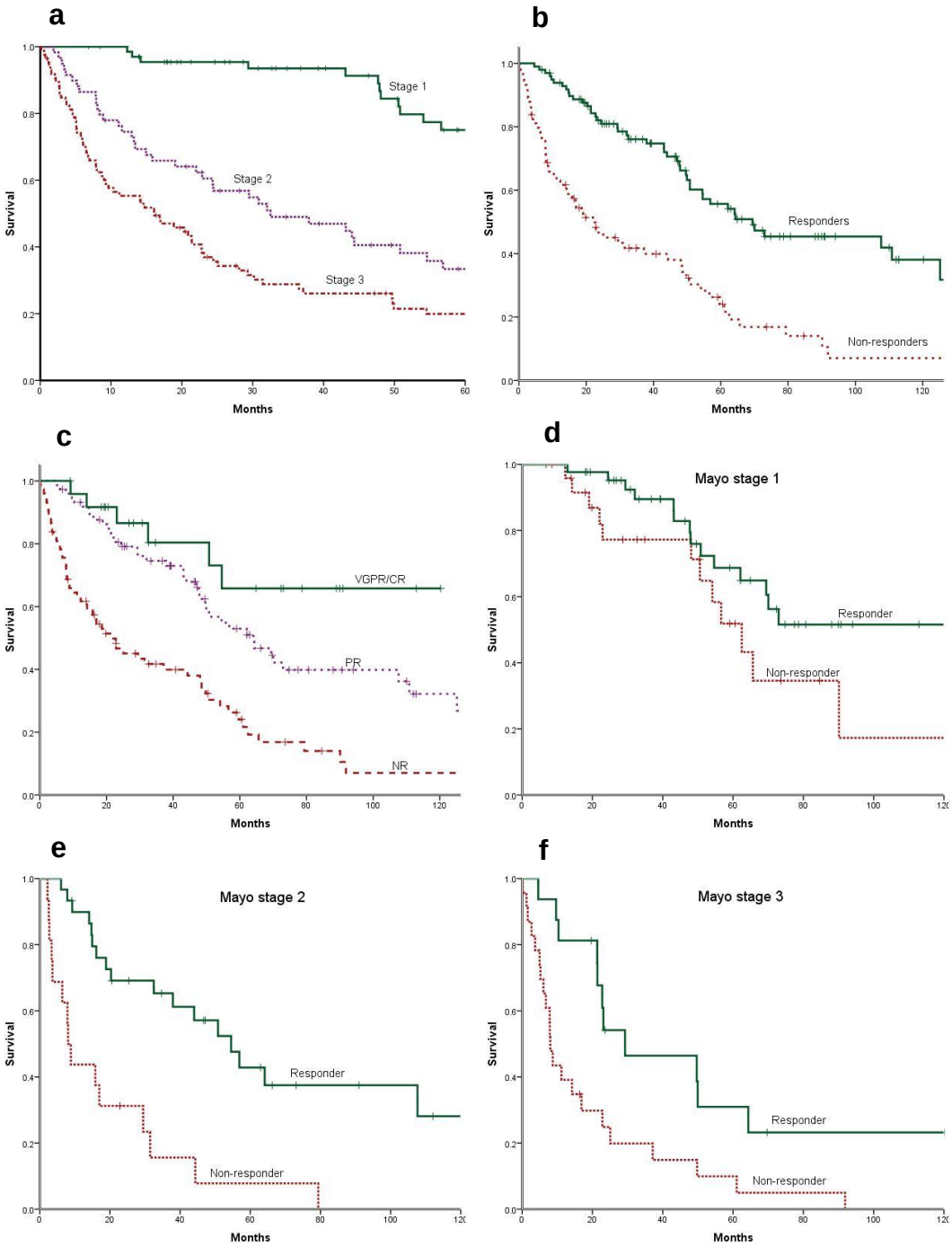
Factor	**Multivariate HR (95%CI); p-values Noise in model:0.04 Harrell's C coef:0.74**	**Multivariate HR (95%CI); p-values Noise in model:0.08 Harrell's C coef: 0.74**
Age (years) (<67 vs ≥67)	1.93 (1.58-2.36); <0.001	2.03 (1.74-2.37); <0.001
Paraprotein ≥10 vs <10	1.23 (0.77-1.97); 0.394	1.50 (1.2-1.87); <0.001
Cardiac vs Non Cardiac	Not included	Not included
Mayo stage	Not included	Not included
Mayo stage I		
Mayo stage II		
Mayo stage III		
Nt-proBNP(ng/l) (≥332 vs <332)	2.99 (2.66-3.37); <0.001	Not included
cTnT ≥0.035 µg/L or cTnI≥0.1µg/L	Not included	3.01 (2.2-4.11); <0.001
Soft tissue vs no Soft tissue	1.35 (0.82-2.2); 0.237	1.42 (1.01-1.99); 0.045
PN vs no PN	1.82 (1.63-2.04); <0.001	2.16 (1.84-2.53); <0.001
AN vs no AN	2.06 (1.92-2.20); <0.001	2.28 (1.84-2.83); <0.001
GI vs no GI involvement	Not included	Not included
Renal vs non Renal	0.92 (0.71-1.20); 0.536	0.93 (0.57-1.51); 0.773
Liver vs non Liver	1.20 (1.10-1.30);<0.001	1.68 (0.94-2.99); 0.077
Albumin (≥30g/l vs <30g/l)	0.59 (0.26-1.32); 0.198	0.59 (0.32-1.07); 0.082
Haematological response vs no response	Not included	Not included

Combining factors independently predictive of survival (NT-proBNP, troponin T, liver involvement and presence of neuropathy), a new risk model is proposed. According to this model, the median survival of patients with none, one or two/more abnormal was 90, 33 and 16 months respectively (Table 5.5) and outlined in figure 5.3a.

Table 5.5 – Shows proposed new prognostic model for IgM-related AL amyloidosis patients[216]

Factors		**Score**
NT-proBNP >332ng/L		1
cTnT >0.035 µg/L or cTnI >0.1µg/L		1
Liver involvement		1
Involvement of PNS and / or ANS		1
Stage	**Score**	**Median OS (months)**
1	0	90
2	1	33
3	2 or more	16

Figure 5.3 a-f, a) Shows the proposed new staging system[216] using - BNP >332ng/L, cTnT >0.035 µg/L or cTnI >0.1µg/L, Liver involvement and Involvement of neuropathy. Stage I – no abnormal features, Stage II – one abnormal feature and Stage III – two or more abnormal features. The median OS for stage I, II and III were 90, 33 and 16 months respectively; **b-f)** Survival by response for entire cohort, by Mayo stage and type of response; **b)** Median OS for those responded to first line treatment - 69 months and for non-responders – 28 months ($p<0.012$); **c)** Median OS for those achieving a VGPR or better was not reached, PR was 64 months and for non-responders was 22 months; **d)** Median OS for responders within Mayo stage I was 134 months and for non-responders was 62 months ($p=0.129$); **e)** median OS for responders within Mayo stage II was 54 months and for non-responders was 8 months, ($p<0.001$) and **f)** Median OS for responders within Mayo stage III was 29 months and for non-responders was 8 months, ($p=0.005$).


a
Stage 1
Stage 2
Stage 3
Survival
Months
b
Responders
Non-responders
Survival
Months
c
VGPR/CR
PR
NR
Survival
Months
d
Mayo stage 1
Responder
Non-responder
Survival
Months
e
Mayo stage 2
Responder
Non-responder
Survival
Months
f
Mayo stage 3
Responder
Non-responder
Survival
Months

Patients who responded to their first line treatment had a significantly better median OS (69 months) compared to the non-responders (28 months) ($p<0.012$) (figure 5.3b). Very good partial response as defined by dFLC remained a predictor of outcome with median OS not reached for patients achieving a VGPR/CR vs. 64 months for those with a PR, ($p=0.183$) and 22 months for non-responders ($p<0.001$) (figure 5.3c). Amongst the patients with only M-protein response, median OS was not reached for responders. Responders within Mayo stage II and III had a significantly better outcome compared to the non-responders, whereas, there was no significant difference within Mayo stage I group, however, the median OS for the responders within this latter group was 134 months and only 62 months for the non-responders (figure 5.3d-f). Median time to next treatment (TTNT) was 12 months with no significant difference when categorised by involved organ (the TTNT for isolated cardiac, renal and liver involvement were 7, 9 and 9 months respectively).

Organ response

On an ITT analysis of organ response, cardiac, liver and renal responses were 3/57 (5%), 7/26 (27%) and 19/108 (18%). Organ response rates are much lower in the IgM cohort compared to that seen in the IgA/IgG-AL cohort in the era of novel agents.[217]

Discussion

Systemic AL amyloidosis associated with IgM-paraprotein is a relatively uncommon variant of AL amyloidosis and accounts for 6% of AL patients.[27] The National Amyloidosis Centre along with the French and Italian groups have previously reported on small series of IgM-AL. These reports have recommended that this sub-group of AL amyloidosis needs to be clearly recognised as a distinct condition and considered for specific treatment targeting the underlying clone.[212, 213, 215, 218] This large series reports the presenting features, response to treatment and clinical outcomes. In addition, it also allowed the identification of novel prognostic factors (neuropathy and liver involvement) unique to this patient population. This study confirms that deeper haematological responses, although still rare in this subgroup, translate into a significant survival advantage.

Since AL amyloidosis is driven by the amyloidogenic light chains, the overall pattern of organ involvement in IgM AL remains broadly similar to that seen in non-IgM AL amyloidosis.[219, 220] The striking difference is the less common cardiac involvement when compared to non-IgM AL amyloidosis (45% vs ~70% respectively).[220] This difference may be due to the relatively lower proportion of lambda light chain isotype in IgM and lower light chain clonal burden. There is a higher incidence of soft tissue and lymph node (35%) involvement, (similar to previous reports[212, 213]) perhaps due to co-existent lymphoma clone at the respective site. The prognostic impact of nerve involvement was unanticipated. Only 13% of patients with nerve

involvement received bortezomib or thalidomide based regimens, raising the question about lack of exposure to novel therapies driving poorer prognosis.

Clear and correct identification of the underlying clonal disorder is key to accurate treatment selection. The underlying clonal disorder is distinctly a non-Hodgkin's lymphoma in 54% of those who had bone marrow biopsy available in this series but plasma cell infiltration is still reported in a proportion (6%) as indeed is the lack of identifiable clonal infiltrate (14%). The latter group possibly indicates that the clone was mostly confined to the lymph nodes with no BM involvement, justifying a lymph node biopsy in such cases. Given the considerable variability in BM reporting as evident above, accurate haematopathology review and use of molecular markers like MYD88 is crucial. The poorer outcome in the group with excess plasma cells, perhaps, lends credence for the use of agents which actively target plasma cells, such as proteasome inhibitors, to be preferentially used in these cases. Cross sectional imaging in IgM AL amyloidosis, particularly to assess lymph node, soft tissue and lung disease, may have an important role. Particularly, in those with lymph node involvement where lymphoid component will respond to treatment but the amyloid may not change – posing a challenge in assessing "true" extent of response. Imaging is important in this condition and its role, including PET-CT, needs clarification.

Contrary to clinical impression and previous publications, 74% of patients in this cohort had abnormal FLC. Patients with either FLC or paraprotein response had improved outcomes. Since, all the patients had a detectable M-protein at a reasonable level, contrary to emerging literature in

non-IgM AL amyloidosis; it may be argued that, in IgM-AL both light chains and paraprotein should be used for response assessment.

Based on previously published smaller series, treatment of patients with IgM-AL has evolved; patients with IgM-AL do not fare well with the "standard" plasma cell directed therapies, not a surprising observation as most cases have an underlying NHL. This series encompasses the changing treatment profiles in this condition. Although a range of regimens were used, rituximab now forms a backbone in most regimens and is used with conventional alkylators (R-CD), purine analogues, bendamustine or with bortezomib with possible resultant improved outcomes. However, the striking paucity of VGPR/CR (14% vs 44% in bortezomib treated non-IgM patients (56% in Mayo stage I cases))[221], highlights the difficulties of achieving deep clonal eradication in low grade NHL. There is a suggestion in this series that patients who achieve a VGPR have much better outcomes than those with lesser degrees of responses – 75% alive at 5 years compared to just over 50% of those with PR. This series validates that the goal of attaining a VGPR/CR still remains the therapeutic end point in patients with IgM-AL, including in those with Mayo cardiac stage II or III disease. Achieving an improvement in organ function is the final goal of therapy. However, the lack of deep clonal responses also translated into paucity in organ responses in this patient cohort compared to non-IgM AL.[222, 223]

Although the median OS in this series is similar to those in previous reports[224], the OS of early stage disease (Mayo stage I and II) in IgM is poorer than non-IgM patients (75% OS at 5 years for stage I vs. >90% in

non-IgM AL[75, 225, 226]); half the expected OS in Mayo stage II patients compared to non-IgM cohort (2 vs ~4 years respectively). Paradoxically, OS of stage 3 appears to be similar when compared to non-IgM-AL possibly due to a lower incidence of very advanced cardiac AL (NT-proBNP >8500 ng/L) in this series and secondly, the lack of a deep clonal response allowing for disease progression. This re-emphasises the need for the development of novel agent based, highly and rapidly effective regimens for this subgroup of patients.

The factors impacting on overall survival are dominated by cardiac involvement, similar to the non-IgM cases. Other poor prognostic factors identified were: older age (>67 yrs.) at presentation, AN or PN involvement, serum albumin <30g/L, dFLC >180mg/l, paraprotein >10g/L, liver involvement and involvement of >2 organs. On multivariate analysis, the independent factors impacting survival were Mayo stage (or abnormal NT-proBNP and troponin), age >67, neuropathy (PN/AN), and liver involvement. The latter two are novel prognostic markers in this group of patients. The adverse impact of liver involvement has been recently demonstrated in Mayo stage I patients.[227] The finding of PN as a significant prognostic factor has important therapeutic implication as proteasome inhibitor, bortezomib appears to be effective and PN may potentially limit its use. A new prognostic staging system for IgM-AL amyloidosis that include presence of neuropathy and liver involvement, is proposed and presented in figure 5.3a (Table 5.5). This finding requires validation with a further study including patients from other major centres.

This study has several limitations including its retrospective nature, small number of patients in each treatment group, lack of detailed haematopathology and imaging for lymphoma diagnosis. Prospective studies in this subgroup of AL amyloidosis are challenging due to the rarity of IgM-AL and difficulty of undertaking studies across national boundaries – wider international collaborative efforts may help to clarify these questions.

In summary, IgM-related AL amyloidosis is a rare and distinct clinical entity of AL amyloidosis. A higher proportion of these patients have lymph node involvement and lower proportion have cardiac involvement. Accurate characterisation of underlying clonal disorder is crucial in the diagnostic work up of patients with IgM-AL. The revised staging system proposed in this disease requires further validation. Striving for VGPR/CR continues to be the primary goal of therapy. Currently, ASCT and bortezomib based regimens seem to be associated with best responses although the prolonged time to next treatment observed with FCR raises the important matter of accurately targeting the lymphoid component of the clone for longer term disease control. Novel targeted therapies need to be further explored in this subgroup of patients. An international tissue and data registry would help to broaden the understanding of this disease.

Chapter Six: Role of ^{99m}Technetium-3,3,-diphosphono-1,2-propanodicarboxylic-acid scintigraphy in patients with light chain (AL) amyloidosis

Introduction

The first three results chapters of the thesis have highlighted the challenging subgroups of AL amyloidosis patients. The clinical profile and treatment outcomes of elderly patients with AL amyloidosis, those with IgM-related and IgD-related amyloidosis have been explored and compared to AL amyloidosis in general whilst highlighting the challenges in these subgroups of patients. The thesis will now focus on novel investigations and prognostic markers which would potentially improve the diagnostic process and help better risk stratify patients and formulate appropriate management plan.

Whilst histological demonstration of amyloid deposition is the gold standard for the diagnosis of amyloidosis, sampling errors, invasive nature of biopsies and procedure related high risk complications are impediments. Moreover, histology cannot provide information on amyloid distribution, extent and disease progression. Conversely, non-invasive imaging offers a better method for assessing extent of amyloid deposition although the numbers of amyloid specific imaging tracers available are limited. It is therefore necessary to develop non-invasive imaging modalities to evaluate

amyloid load, quantify and monitor disease progression and response to treatment. Much progress has occurred in the development of non-invasive imaging methods over the last decade. These include serum amyloid P component (SAP) scintigraphy, cross sectional computerised tomography (CT), positron emission tomography (PET) tracers, cardiac magnetic resonance imaging (CMR) and a number of bisphosphonate bone tracers. ^{123}I-SAP scintigraphy has been in routine clinical use at the National Amyloidosis Centre for over two decades for visceral imaging but is unable to image amyloid deposits in the heart, lungs, nerves or soft tissues.[80] The bisphosphonate tracer, ^{99m}Tc-DPD has been identified as one of the most sensitive methods of imaging cardiac amyloid deposits in transthyretin (ATTR) amyloidosis.[97]

As yet, there are no reported modalities for specifically imaging soft tissue amyloid deposits, and hence, diagnosis is usually based on biopsy of the suspicious lesion if this is deemed safe.

^{18}Fluorine labelled fluorodeoxyglucose-positron emission tomography (FDG-PET) has been reported to be positive in patients with localised AL amyloidosis[228] – the reason for the FDG uptake remains unclear but has no relationship to the amyloid fibrils per se and is due to either the infiltration of monoclonal B cells or the cellular tissue reaction to the amyloid fibrils.

The utility of ^{99m}Tc-DPD for imaging soft tissue amyloid deposits has never been fully reported. This chapter reports on the specific uptake of the bisphosphonate bone tracer, ^{99m}Tc-DPD by amyloid deposits in soft tissue, lymph nodes (LN) and lung parenchyma.

Methods

Patient selection

The study was performed at the UK National Amyloidosis Centre (NAC) and included all patients with localised amyloidosis who underwent ^{99m}Tc-DPD scintigraphy between 2010 and 2015. All patients assessed had routine ^{123}I-SAP scintigraphy for assessment of visceral amyloid deposits and ^{99m}Tc-DPD scintigraphy for cardiac amyloidosis.

^{99m}Tc-DPD Scintigraphy

Patients were scanned as previously described, using General Electric Medical Systems hybrid gamma cameras (Infinia Hawkeye 4 and Discovery 670) following the intravenous injection of 700 MBq of ^{99m}Tc-DPD.[206] In brief, whole body planar images were acquired three hours post-injection followed by SPECT-CT (single photon emission computed tomography with a low-dose, non-contrast CT scan) at the site of ^{99m}Tc-DPD uptake.

Results

A total of twenty six patients were included in this study. All 26 patients had extra-cardiac uptake on ^{99m}Tc-DPD scintigraphy and none had cardiac uptake. These were confirmed on SPECT/CT. Table 6.1 summarizes the baseline characteristics for these patients. Using the ICC, one patient had

cardiac involvement, two had liver involvement and one had macroglossia. On cross-sectional imaging the extra-cardiac organs involved were: LNs in 17 (65%) (Figure 6.1a), breast in three (12%) (Figure 6.1b), skin/subcutaneous soft tissue in three (12%) (Figure 6.1b) and lung in five (19%) (Figure 6.1c). All 26 patients had biopsy proven amyloid deposits. 23/26 (88%) had a biopsy taken from the site of extra-cardiac uptake confirming amyloid deposition. The underlying clonal dyscrasia was plasma cell in 14 and IgM producing lymphoma in seven patients.

Table 6.2 shows the distribution of uptake on ^{99m}Tc-DPD scintigraphy for the 26 patients. Five had visceral uptake on ^{123}I-SAP scintigraphy (liver and spleen in one and spleen in four), however none of these patients showed corresponding ^{99m}Tc-DPD uptake in the liver or spleen.

Table 6.1 Baseline characteristics of twenty six patients with extra-cardiac uptake on ^{99m}Tc-DPD scintigraphy.

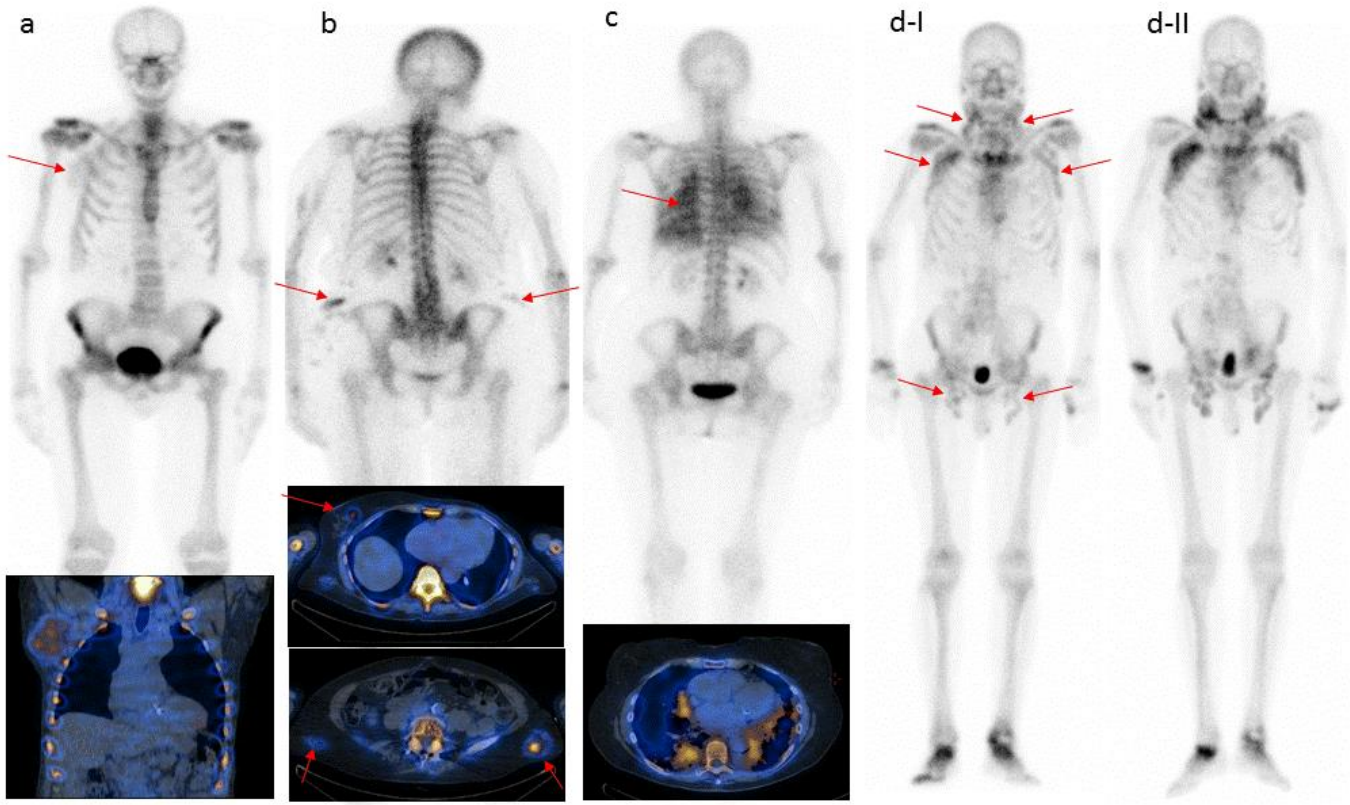
Variables	Total number (%)/Median(range) (n=26)
Male: Female ratio	1.36:1
Age (years)	73.5 (49.2-86.9)
Confirmation of amyloid deposition Breast tissue LN Lung parenchyma / Pleural Fat aspirate Orbit tissue	26 (100%) 3 (12%) 17 (65%) 5 (19%) 1 (4%) 1 (4%)
Type of underlying clonal disorder IgA IgG IgM Light chain only	21 (85%) 1 (4%) 10 (38%) 7 (27%) 3 (12%)
Presenting paraprotein level (g/L)	12 (IF – 26)
Involved light chain – Kappa: Lambda	11:15
Organ involvement	
Cardiac	1 (4%)
Renal	0
Liver	2 (8%)
Neuropathy	2 (8%)
Systemic vs Local AL	8 (19%) vs 18 (69%)
NT-proBNP (pmol/L)	31.5 (4-668)
Troponin T (µg/L)	10 (3-98)
Mayo stage biomarkers 1 2 3 Missing	 12 (46%) 9 (35%) 1 (4%) 4 (15%)
Creatinine clearance (mls/min)	65.5 (22.4-166)
Albumin (g/L)	43 (31-47)
ALP	77 (13-193)

Table 6.2 Involvement by amyloid deposits and distribution of uptake on ^{99m}Tc-DPD scintigraphy

No	Sites	Uptake on ^{99m}Tc-DPD scintigraphy	^{123}I SAP uptake
1	LN	Axillary, cervical and hilar nodes	Nil
2	LN	Left hilum, subcarinal and para-tracheal nodes	Nil
3	LN	Hilar and para-tracheal nodes	Nil
4	LN	Mediastinal and para-tracheal nodes	Nil
5	LN	Mediastinal mass, mediastinal nodes and pericardial uptake	Nil
6	LN	Bilateral axillary, right supraclavicular and mediastinal nodes	Nil
7	Lung parenchyma	Diffuse parenchymal lung involvement in association with likely pleural involvement	Nil
8	LN	Axillary node	Nil
9	Breast, soft tissue	Bilateral breast, lower limb and pelvic girdle	Nil
10	Soft tissue	Soft tissue within right leg	Nil
11	LN	Right axillary node	Nil
12	Breast	Soft tissue deposits within the subcutaneous fat	Nil
13	LN	Cervical, sub-pectoral, supraclavicular, retroperitoneal and pelvic nodes	Nil
14	Lung parenchyma	Soft tissue masses within the thorax	Nil
15	Lung parenchyma	Pulmonary nodules	Nil
16	Lung parenchyma	Pulmonary nodules	Nil
17	LN	Mediastinal nodes	Nil
18	Lung parenchyma	Bilateral lung fields	Nil
19	LN, Liver, bile duct	Axillary, mediastinal, retroperitoneal, mesentery, inguinal and pulmonary nodules and hepatic parenchyma	Spleen
20	LN, Breast, Skin	Breast, soft tissue, lymph node	Nil
21	LN	Lymph nodes above and below diaphragm	Spleen
22	LN, Cardiac, Liver, PN	Para-tracheal, pre-carinal, retrocrural and para-oesophageal nodes	Spleen, Liver
23	LN, Macroglossia	Cervical, supraclavicular, bilateral axillary and mediastinal nodes	Nil
24	LN	Inguinal nodes	Spleen
25	LN	Left inguinal, left external iliac, common iliac and retroperitoneal nodes	Spleen
26	LN, PN	Bilateral Cervical and axillary nodes	Nil

The median duration of follow-up was 23.45 months (range 0.93 – 104.8 months). One of the 17 patients with LN uptake had repeat ^{99m}Tc-DPD scintigraphy 25 months later which showed evidence of progressive lymph node involvement, demonstrating significantly increased intensity and size of the abnormal foci of tracer uptake in the same distribution as well as new nodal involvement (Figure 6.1d).

Figure 6.1a-d Figures demonstrate images of ^{99m}Tc-DPD uptake in four AL patients, by a) Lymph node, b) breast and skin, c) Lung parenchyma and d-I) Lymph nodes at baseline and d-II) 25 months later showing progression.



Discussion

Whilst cross-sectional imaging will show abnormal tissue deposition, it lacks specificity for amyloidosis. There are no imaging modalities that provide information on the extent of disease in localised amyloidosis. Identification of amyloid deposits in soft tissue and small sites such as LNs are mainly from biopsy confirmation of the affected sites.

Myocardiac uptake of bone tracers, particularly DPD, and other bone tracers, is well known. An abundant literature exists on the subject, attributing such uptake to various causes, but first and foremost to cardiac transthyretin (TTR) amyloidosis.[229, 230] In addition, sparse reports of soft tissue uptake in bone scans have been published along with possible mechanisms for the phenomenon.[231-233] De Haro *et al*[234] reported tissue uptake of ^{99m}Tc-DPD in a patient with biopsy proven systemic AL amyloidosis. Tracer uptake was seen in the heart, thyroid, parotid glands, uterus and intestinal tract.[234] Reports have also described soft tissue uptake by the liver, heart, skeletal muscle, and splenic uptake by ^{99}Tc-MDP and ^{99}Tc-PYP scans of AL amyloidosis patients. [235, 236] Itoh et al in 1992 reported a case of primary amyloidosis with calcification of systemic lymph nodes which were demonstrated as positive by bone scintigraphy.[237]

This study reports the important findings of extraosseus and extra-cardiac uptake of bone tracers on ^{99m}Tc-DPD scintigraphy in 26 patients with AL amyloidosis involving various sites. SPECT-CT images can help precisely delineate the non-osseous uptake and identify calcium content of the site. What was striking in these parties was the lack of cardiac uptake

even though all demonstrated tracer uptake in sites other than the heart. The sites showing increased uptake were predominantly LN but also included breast tissue, lung parenchyma and muscle.

Seventeen patients had LN uptake at various sites. LN involvement either isolated or as part of systemic amyloidosis is currently imaged with CT or PET-CT scans both to document the extent of the disease and assess any response to treatment. ^{99m}Tc-DPD scintigraphy also may have a role in detecting the extent and distribution of LN amyloidosis and differentiating this from lymphomatous infiltration.

Pulmonary AL amyloidosis is rare and can potentially present in five different forms: Diffuse interstitial or alveolar-septal disease, nodular, intra and extra-thoracic adenopathy, pleural disease and diaphragm deposition.[238] It is difficult to diagnose - patients often present with nodules which need invasive biopsy (often surgical). Five patients with AL amyloidosis proven on lung parenchymal tissue had tracer uptake involving lung fields or pulmonary nodules on ^{99m}Tc-DPD scintigraphy. ^{123}I-SAP scintigraphy is not able to provide images of amyloid deposits in diffuse organs such as the lungs. CT is the most frequently used imaging modality in amyloidosis confined to the respiratory tract, providing quantitative assessment of airway narrowing and extent of disease locally. Diffuse pulmonary amyloidosis is better identified on high-resolution CT which is also useful in monitoring the disease course with the help of serial pulmonary function tests. Our findings suggest that ^{99m}Tc-DPD would complement CT in the diagnosis of pulmonary AL amyloidosis involving the respiratory tract.

AL amyloidosis of the breast is an unusual diagnosis and has been reported by several groups and account for 0.5% of all patients referred to amyloid treatment centres.[239] Amyloidosis of the breast may present as a distinct lesion or intermixed with breast cancer in about 50% of cases.[240] Patients rarely experience any clinical symptoms and the initial findings are noted on a mammogram or an ultrasound. Three of the patients from this study with AL amyloidosis proven on breast tissue biopsy had ^{99m}Tc-DPD uptake in the affected breast. One of these patients had systemic amyloidosis and the other two had localised form. Whilst ^{99m}Tc-DPD scintigraphy is not a substitute to the current investigative modalities of breast lesions, it can certainly play a role in confirming the diagnosis of breast tissue amyloidosis in affected patients complementing the existing techniques.

Pathologic conditions which may lead to a soft-tissue accumulation of diphosphonate and pyrophosphate have been reviewed by Brill *et al*.[241] However, all the reports so far have been in liver, spleen and skeletal muscle. This is the first series reporting uptake in LNs, breast and lung tissue. The mechanism of ^{99m}Tc-DPD soft tissue or LN uptake in light chain amyloid patients is not clear. The binding of radionuclide labelled calcium seeking agents, may be explained by the high calcium content of amyloid. The result obtained by nuclear bone scans most likely depends on the type of calcium-seeking agent used and the amyloid content at the site.[242] The failure to demonstrate ^{99m}Tc-DPD accumulation does not exclude the possible presence of amyloid. In order to understand this phenomenon

further, a directed biopsy of lesions with significant soft tissue uptake by the agent suggesting the presence of amyloid deposits is vital.

Other imaging techniques such as ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography-CT (PET-CT) scans showing FDG avidity at known disease sites of some localised AL amyloidosis are also gaining popularity.[228] Recently, a phase I study using murine IgG1 mAb 11-1F4 labelled with ^{124}Iodine reported 18 patients with AL amyloidosis in which fifty per cent of the patients showed uptake in liver, lymph nodes, bone marrow, intestine or spleen (but not kidneys or heart).[102]

In summary, moderate to intense uptake of radiolabelled bone tracers by LN, lung and breast tissue amyloid have not been widely recognised. ^{99m}Tc-DPD scintigraphy is a useful imaging modality to detect soft tissue, particularly, LN, breast tissue and lung parenchymal amyloid and may also be useful for serial imaging. This technique is particularly useful in patients with IgM related AL amyloidosis in which soft tissue amyloidosis accounts for 35% of patients of whom 20% have LN amyloidosis.[216] It also has a role in complementing the current diagnostic modalities in patients presenting with localised AL amyloidosis involving soft tissue such as breast and pulmonary amyloidosis.

Chapter Seven: The prognostic role of multicolour flow cytometry in AL amyloidosis

Introduction

It is very well established that the prognosis of AL amyloidosis is very much dependent on the extent of organ damage, mostly determined by cardiac involvement. In the last decade, there has been an improvement in the overall survival of patients with systemic AL amyloidosis following the advent of novel anti-plasma cell agents. However, there continues to be a subgroup of patients with advanced AL amyloidosis with a dismal outlook despite the availability of many therapeutic agents and better supportive care. Lately, the impact of plasma cell clone on outcomes has become a focus of interest since the treatment is to eliminate the underlying clonal PCs following the principles of treatment in multiple myeloma.

The Mayo cardiac staging has remained the most widely used and clinically relevant prognostic system in AL. Additionally the level of the amyloidogenic precursor, the serum free light chain level, has also been incorporated in the staging system. However, the final determinant of outcomes in AL is the actual biologic characteristics of plasma cell clone which governs the sensitivity to treatment, duration of response after treatment and development of clonal resistance – all of which are well studied in symptomatic myeloma but remain to be fully explored and understood in AL amyloidosis. The Mayo group also recently showed that

patients with an absolute bone marrow plasma cell (BMPC) percentage greater than 10% had inferior outcomes compared to those with lower percentages – outcomes of the former similar to symptomatic myeloma.[243] In monoclonal gammopathy and smouldering myeloma, determination of proportion of the 'normal' and clonal plasma cells in a bone marrow sample has prognostic significance.[244] Paiva *et al* recently reported in a small series that AL patients with more than five percent 'normal' BMPC (defined as cells expressing CD38+CD138+CD19+) at diagnosis had a better prognosis.[119] Since then, a study by the Mayo group, reported on the prognostic role of multicolour flow cytometry (MFC) in AL amyloidosis at diagnosis and at the end of treatment. They also concluded that MFC may have a role in defining haematological response.[245]

This study explores the impact of bone marrow plasma cell burden on outcomes in systemic AL amyloidosis, using both standard morphological techniques to determine plasma cell percentages as well as proportion of 'normal' plasma cells as determined by MFC.

Methods

Patient selection

This study included all patients with newly diagnosed systemic AL amyloidosis, seen at the UK National Amyloidosis Centre between 2005 and 2013. All patients included in this study were required to have had both bone marrow trephine (BMT) and MFC performed at presentation either at the UK National Amyloidosis Centre or at the Haematological Malignancy diagnostic

service, St James's University Hospital in Leeds during the study period. The plasma cell burden was morphologically estimated as previously described.

Outcome measures and Statistics

Primary outcome measures studied was overall survival. Statistical analysis was undertaken using the SPSS 21 software package (SPSS, Chicago, IL). Survival was assessed by the method of Kaplan and Meier and compared by log-rank test. Categorical variables were compared with chi-square or Fisher's exact tests as appropriate. All *P* values were 2 sided with a significance level of 0.05. ROC analysis was undertaken to identify cutoffs for proportion of 'normal' vs. aberrant plasma cells by MFC. Multivariate analysis was by Cox or binary logistic regression as appropriate.

Results

There were 103 patients with biopsy proven systemic AL amyloidosis with bone marrow trephine biopsy results and MFC performed on bone marrow aspirates. The median age was 64.7 years (range: 38.5-83.3) with a male-female ratio of 1.6:1. Sixty-three (61%) had cardiac involvement. Table 7.1 shows patient characteristics at presentation. BMT was inadequate for three patients. The median plasma cell percentage was 15% (range 2-90%) for the remaining 100 patients. Fifty five patients (55%) had ≥10% PCs on trephine (classed as AL-MM) and 45 (45%) had <10% (classed as AL-MGUS). All patients had MFC and the median total plasma cell on MFC was 1.100%

(range: 0.017-12.630). All patients had aberrant plasma cells on MFC. The median neoplastic PCs were 96% (range: 9.82-100%). The median normal PCs were 4.00% (range 0-72.57%). ROC analysis identified presence of ≥10% normal PC as a proportion of total plasma cells in a BMT sample as a significant cut-off for survival outcomes. Thirty (29%) patients had ≥ 10% normal PCs on MFC and 73 (71%) had <10% normal PC. 21/30 (70%) of those with ≥10% normal PC by MFC had been reported as having AL-MGUS by morphology on BMT. There was a statistically significant negative correlation between the plasma cell percentage of BMT and the normal PC percentage on MFC (Spearman correlation -0.394, p=0.004).

Table 7.1 Patient characteristics at presentation.

Patient characteristics	**Number of patients (%) / Median (range)**
No. of patients	103
Sex (Male: Female)	1.6:1
Age at presentation (range)	64.7 (38.5-83.3)
Monoclonal protein type IgG IgA IgM IgD Light chain only	 31 (30%) 9 (9%) 2 (2%) 1 (1%) 60 (58%)
Paraprotein concentration (g/L)	12 (IF-23)
Involved free light chain type Kappa (mg/L) Lambda (mg/L)	 32 (31%) / 120 (21-9290) 71 (69%) / 190 (26.8-2940)
Baseline involved FLC (mg/L)	170 (21-9290)
Baseline dFLC (mg/L)	169 (0.20-9280)
Organ involvement	
Cardiac	63 (61%)
Systolic blood pressure (mmHg)	100 / 118 (80-178)
Systolic blood pressure <100 mm of Hg	17 (17%)
NT-proBNP (ng/L) BNP ≥332ng/L BNP ≥8500ng/L	102 / 2127 (34-46373) 79 (77%) 21 (21%)
TnT ng/ml	80 / 0.057 (0.005 – 0.73)
Mayo Stage	n= 80 (78%)
I II III	16 (20%) 29 (36%) 35 (44%)
LVS (mm)	83 / 13 (7-21)
Renal	82 (80%)
Serum creatinine (μmol/L)	101 / 87 (36-781)
Urinary protein (g/24hrs)	96 / 3.10 (0-18)
Albumin	100 / 34 (16-50)
Liver by consensus criteria	25 (24%)
Alkaline phosphates (U/L)	101 / 85 (19-1347)
PNS	11 (11%)
ANS	15 (15%)
Soft tissue	18 (18%)
MGUS vs MM	43:57
Alive / dead	50:53

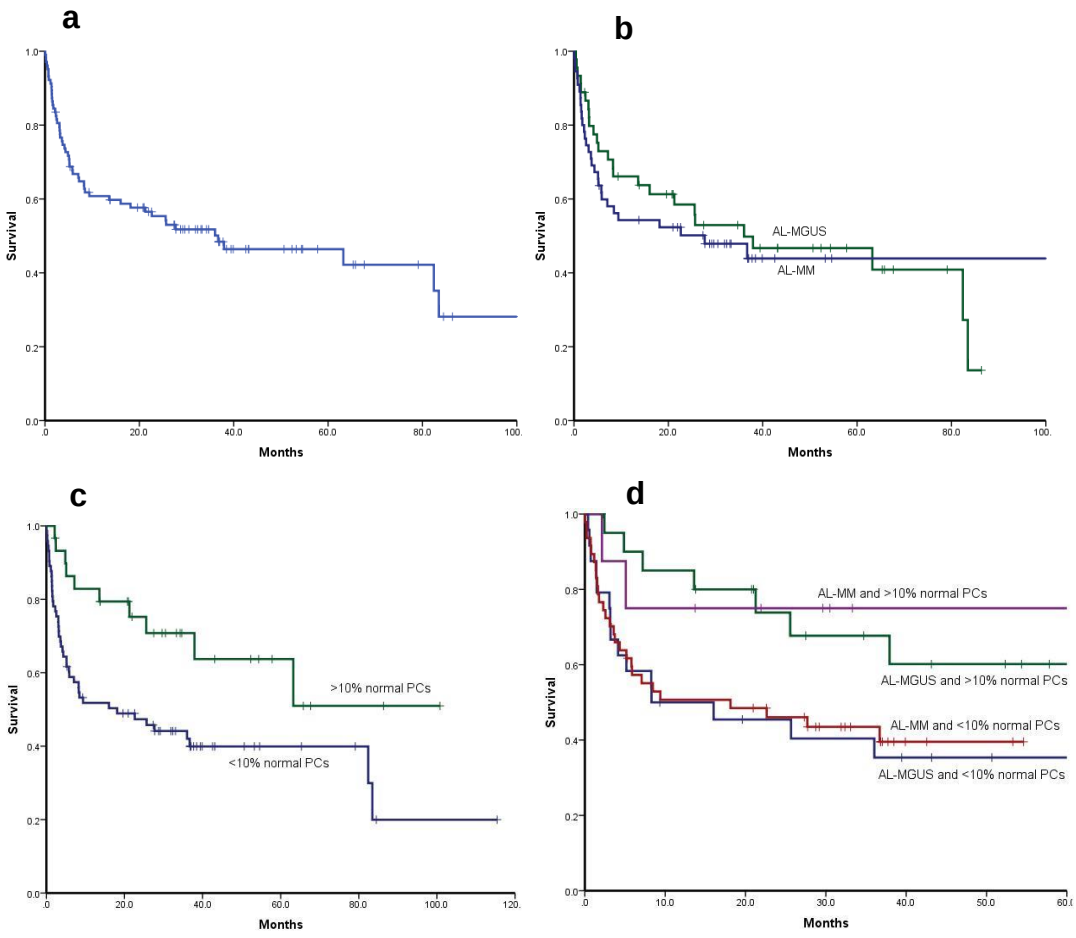
There was a higher proportion of renal involvement in the ≥10% 'normal' PCs group (28/30 (93%) vs. 54/73 (74%); p=0.031) whilst the converse was true for cardiac involvement. There was a significant negative correlation between ≥10% normal PCs on MFC and cardiac involvement (Spearman correlation -0.454, p <0.001). Only 8/30 (27%) patients with ≥10% normal PC by MFC had cardiac involvement compared to 55/73 (75%) of those with <10% normal PCs (p <0.001). The group with ≥10% normal PCs also had a significantly lower number of patients with systolic blood pressure (SBP) <100mmHg (1/30 vs 16/70; p=0.017) and lower NT-proBNP (median 589ng/L vs. 3288ng/L, p=0.001), surrogate markers of advanced cardiac involvement. The median dFLC in patients with ≥10% normal-PC was 38 mg/L compared to 233 mg/L for those with <10% normal PC (p<0.001). Of the patients with dFLC of <180mg/L, 24 had ≥10% normal PC and 27 had <10% normal PC; for those with dFLC ≥180mg/L, the numbers of patients with ≥ or < 10% normal PC were 6 and 45 (p<0.001) respectively. The former group therefore had lower number of patients with dFLC≥180mg/L (6/51, p<0.001).

Survival Outcomes

The median overall survival (OS) for the whole cohort was 36.6 months. The morphological percentage of plasma cells identified by BMT had a non-significant impact on OS: AL-MGUS - 36 months and AL-MM - 27.7 months (p=0.605). Patients with ≥10% normal PCs on MFC had a significantly better survival (median OS not reached) compared to those with <10% normal PCs (18.1 months) (p = 0.012) (figure 7.1a-c). We assessed the impact of < or

≥10% normal PCs by MFC in patients with morphologically determined AL-MGUS or AL-MM. The median OS of patients with AL-MGUS by BMT with ≥10% normal PC on MFC was 63.2 months and <10% normal PCs was 8.3 months (p=0.038) with estimated five year survival 60% and 34% respectively. Median OS of patients with AL-MM by BMT with ≥10% normal PC by MFC was not reached compared to 18.1 months for those with <10% normal PC (p=0.151) with estimated five year survival 75% and 37% respectively (Figure 7.1d). Within the respective ≥10% and <10% normal PC groups on MFC, there were no significant difference in the overall survival of those with AL-MM and AL-MGUS by BMT (p=0.75 and p=0.81 respectively).

Figure 7.1a-d Shows a) Overall survival of the whole cohort (36.6 months); **b)** the median OS of patients with AL-MGUS (36 months) and AL-MM (27.7 months) (p=0.605), based on the morphological percentage of plasma cells identified by BMT; **c)** the median OS of patients with ≥10% normal PCs (median OS not reached) compared to those with <10% normal PCs (18.1 months) (p = 0.012) on MFC; **d)** the median OS of patients with AL-MGUS by BMT with ≥10% normal PC on MFC (63.2 months) and <10% normal PCs (8.3 months) (p=0.038), median OS of patients with AL-MM by BMT with ≥10% normal PC by MFC (not reached) and those with <10% normal PC (18.1 months) (p=0.060)



The impact of organ involvement and overall survival stratified by presence of ≥10% normal PCs or <10% normal PCs is shown in table 7.2.

Table 7.2 The impact of organ involvement and overall survival stratified by presence of ≥10% normal PCs or <10% normal PCs

Organ involved	**No of patients categorised by % of PCs on MFC (%)**			**Median survival by Kaplan Meir (months) categorised by % of PCs on MFC - with and without factor (*P* value)**	
	≥10%	**<10%**	***P* value**	**≥10%**	**<10%**
Cardiac	8/30 (27%)	55/73 (75%)	<0.001	25.6 vs NR (0.180)	5.8 vs NR (<0.001)
SBP <100mmHg	1/30 (3%)	16/70 (23%)	0.017	5.1 vs NR (0.022)	4.1 vs 25.7 (0.193)
NT-proBNP ≥332ng/L	19/30 (63%)	60/72 (83%)	0.028	37.9 vs NR	7.1 vs NR (0.003)
dFLC ≥180mg/L	6/30 (20%)	45/72 (63%)	<0.001	13.6 vs NR (0.002)	5.9 vs 82.4 (0.030)
Renal	28/30 (93%)	54/73 (74%)	0.027	NR vs 5.1 (0.360)	25.7 vs 5.2 (0.137)
NT-proBNP ≥8500ng/L	4/30 (13%)	17/72 (24%)	0.242	2.4 vs NR (0.048)	3.1 vs 82.4 (<0.001)
Mayo Stage I II III	9/21 (43%) 9/21 (43%) 3/21 (14%)	7/59 (12%) 20/59 (34%) 32/59 (54%)	0.001	Too few patients for survival analysis	5.1 vs NR vs NR (0.001)
Liver	12/30 (40%)	16/73 (22%)	0.061	NR vs 63.2 (0.336)	4.1 vs 11.1 (0.686)
PNS	3/30 (10%)	8/73 (11%)	0.886	Too few patients for survival analysis	3.7 vs 18.1 (0.886)
ANS	3/30 (10%)	12/73 (16%)	0.386	NR vs NR (0.856)	3.1 vs 27.7 (<0.001)
Soft tissue	3/30 (10%)	14/73 (19%)	0.244	2.4 vs NR (<0.001)	5.2 vs 18.1 (0.765)
Haematological response	13/21 (62%)	37/67 (55%)	0.590	NR vs 13.6 (0.002)	82.4 vs 2.3 (<0.001)

The BMPC did not have any bearing on the outcome of patients with either cardiac or non-cardiac involvement. Patients with cardiac involvement and 'normal' PC ≥10% on MFC, had slightly superior outcome with median OS 25.6 months compared to only 5.8 months in those with <10% 'normal' PCs, however, this was not statistically significant (p=0.291) and the number of patients in the former group was only eight, compared to 55 in the latter group. In patients without cardiac involvement, neither BMPC burden on BMT by morphology nor the proportion of 'normal' PCs on MFC influenced outcome (Figure 7.2a). Patients with dFLC≥180mg/L had median OS of 13.6 months when 'normal' PC was ≥10% compared to only 5.9 months in those with PC <10% on MFC (p=1.00). However, there was a noticeable impact of 'normal' PCs on survival of those patients with dFLC<180mg/L at presentation with median OS 82.4 months in those with <10% 'normal' PCs and 'not reached' in those with ≥10% 'normal' PCs (p=0.072).

Treatment

Ninety six patients received chemotherapy and the details of the regimen were not available for two of these patients. One died before receiving therapy and four patients chose not to receive chemotherapy. The details of first line regimen used in the 94 patients are shown in table 7.3. The presenting free light chains and paraprotein were too low for evaluation in eleven patients. Eighty-eight patients were therefore, included in the

intention to treat analysis, of whom, 50 (57%) had achieved a haematological response (24% CR, 16% VGPR and 17% PR).

There was no significant correlation between haematological response and the proportion of normal PC by MFC. However, patients who had not responded to first line therapy with ≥10% 'normal' PCs had a superior survival with a median OS of 13.6 months compared to only 2.3 months in those with <10% 'normal' PCs (p=0.093). The median OS for patients with a haematological response and ≥10% 'normal' PCs was 'not reached' and that of those with <10% PCs was 82.4 months (p=0.203) (figure 7.2b). The BMPC as established on trephine biopsy did not influence the outcome within the responders and non-responders groups.

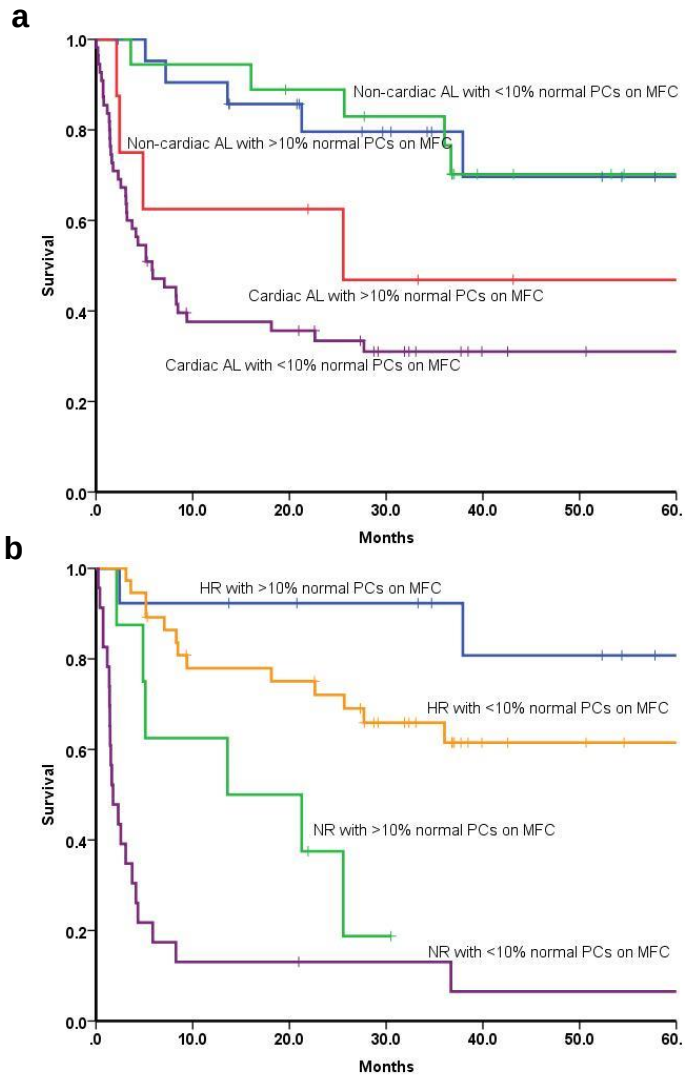
The factors significantly impacting on survival on univariate analysis were, <10% 'normal' PCs on MFS, cardiac involvement, advanced Mayo stage, autonomic nervous system involvement, SBP <100mmHg, NTproBNP ≥332ng/L, NTproBNP ≥8500ng/L, dFLC≥180mg/L, non-renal involvement and haematological response to treatment.

dFLC was excluded from multivariate models due to a correlation between dFLC and </≥10% normal PCs. Mayo stage alone at baseline (Hazard ratio (HR) 20.82 (95% CI 2.82-154.01); p=0.003) and Mayo stage (HR 10.17 (95% CI 1.15-90.12); p=0.037), Haematological response (HR 6.53 (95% CI 1.62-26.36); p=0.008) and <10% normal PCs (HR 5.18 (95% CI 0.584-45.94); p=0.143) in landmark analysis were independent factors impacting survival on multivariate analysis.

Table 7.3 Details of first line regimen used in the 94 patients

Regimen	**No. of patients (%)**
ASCT	1 (1%)
Thalidomide	44 (47%)
Bortezomib	33 (35%)
Alkylating agents (Melphalan / Cyclophosphamide)	14 (14%)
Lenalidomide	1 (1%)
VAD	1 (1%)

Figure 7.2a-b Shows **a)** Survival by cardiac involvement and proportion of normal PC by MFC - Patients with cardiac involvement and 'normal' PC≥10% on MFC, (median OS 25.6 months), cardiac involvement and 'normal' PC<10% on MFC (5.8 months) (p=0.291, SE: 1.91, CI: 2.12-9.6), patients without cardiac involvement and 'normal' PC≥10% on MFC (median OS not reached) and patients without cardiac involvement and 'normal' PC<10% on MFC (median OS not reached) and **b)** survival by haematological response and proportion of normal PC by MFC – Non responders to first line therapy with ≥10% 'normal' PCs (13.6 months), <10% 'normal' PCs (2.3 months), Haematological responders with ≥10% 'normal' PCs (median OS not reached) and <10% PCs (82.4 months) (p=<0.01).



Discussion

The management of patients with AL amyloidosis follows a risk stratified approach. The end organ damage caused by the amyloid fibrils, particularly to the heart, dominates this algorithm. Lately, the importance of bone marrow plasma cell infiltration and its clonal characteristics is being increasingly factored into planning therapy for patients. This chapter highlights the importance of using MFC to characterise the nature of plasma cells in bone marrow and show that the proportion of normal to abnormal plasma cells is a key factor in determining prognosis; not just a morphological estimation of total number of plasma cells in the bone marrow biopsy.

The characteristic of the patient population studied here is very similar to that previously reported in AL patients in general but our study consisted of a slightly higher proportion of patients with cardiac amyloidosis. The level of plasma cell infiltration in a patient with AL amyloidosis is generally lower than that of multiple myeloma with a reported median percentage of plasma cells being ~7-10% with 38 % having a ≥10% plasma cells infiltration (AL-MM category).[27, 117, 121, 243] The maximum percentage of total plasma cells on MFC was only 12.63% (compared to 90% by morphology/trephine). Flow cytometry has limitations in estimation of true marrow infiltration by BMPC due to sample dilution effect.

An important study from the Mayo group reported that patients with AL-MGUS (<10% BMPC by morphology) had significantly superior outcome compared to those with ≥10% BMPC by morphology even in absence of

symptomatic myeloma – the latter outcomes similar to that of patients with AL amyloidosis who had symptomatic myeloma (median OS 46, 16.2 and 10.6 months respectively).[243] This study raised important questions about planning therapy in patients with AL-MM category – whether to follow the AL guidelines (often consisting of shorter duration of therapy and autologous stem cell transplantation is not always considered) or use the standard myeloma treatment algorithms with more aggressive therapy. The current study however, does not support the findings from the Mayo study, perhaps due to the relatively smaller cohort of patients in this study. The median OS was three years for the whole cohort with no significant difference between the OS of those with AL-MGUS and AL-MM; although, the former had a slightly superior outcome. It is possible that this may reach significance with larger patient numbers.

Studies on myeloma and MGUS without amyloidosis have defined an important role for multiparameter flow cytometry in characterisation of the plasma cells in the bone marrow. Suppression of the normal plasma cells by the malignant or aberrant plasma cell clone is likely to be an important feature of clonal “aggressiveness” in plasma cell dyscrasias. It appears that there is progressive competition for overlapping bone marrow niches, which leads to replacement of normal BM cells by clonal plasma cells and associated with more advanced disease in patients with MGUS, smouldering and symptomatic myeloma.[246] Patients with <5% normal bone marrow plasma cells in MGUS have significantly higher risk of progression to myeloma and the proportion of residual normal plasma cells was a stronger prognostic factor than conventional markers for progression in both SMM

and MGUS.[244, 247] Aberrant expression of various antigens on plasma cells have been reported in patients with AL amyloidosis, which, have helped in the identification of neoplastic PCs in AL patients with low volume disease burden.[22, 248, 249] Most recently, the Spanish group have extended their method of normal vs. aberrant plasma cells in the bone marrow to patients with AL amyloidosis using the same (5% normal plasma cell) threshold.[119] They reported that patients with less than 5% normal BMPC had a two year survival rate of 88% compared to 37% in those with more than 5% BMPCs.[119] This study confirms the previous report of the survival advantage of 'normal' PCs on MFC. However, in this study, using ROC analysis, we identified 10% normal BMPC as the threshold best for defining prognosis in AL amyloidosis rather than the lower cut off of 5% that was used in the Spanish Study. Patients with ≥10% 'normal' PCs on MFC had a significantly better survival than those with <10% 'normal' PCs (median OS - not reached vs. 18.1 months respectively, p=0.012), regardless of the BMPC burden on trephine. Patients with AL-MM (with ≥10% plasma cells by morphology on BMT) had a better outcomes if they had ≥10% 'normal' PCs by flow cytometry. More interestingly, this observation was true for those patients with a <10% plasma cell infiltration by morphology on BMT (AL-MGUS). The outcome of patients with AL-MGUS with <10% 'normal' PC on MFC was significantly worse than those with ≥10% 'normal' PCs (median OS 8 months vs. ≥5 years, p=0.038) with a doubled five year survival for the latter group.

The percentage of normal or aberrant PC in BM by flow cytometry also correlated with other markers of disease burden in AL amyloidosis. High serum free light chains (the causative culprit in AL amyloidosis)

correlate directly with outcomes – patients with a dFLC of ≥180mg/L have poorer outcomes.[121] However, there has never been a formal study correlating bone marrow findings with the level of light chains. In the current study, similar to the established criteria, patients with dFLC of <180mg/L had superior outcomes. There was a significantly greater proportion of patients with ≥10% normal PC in the cohort with low (<180mg/L) dFLC compared to those with higher values. With both cohorts (those with dFLC of <180mg/L and ≥180mg/L), the proportion of residual normal PC ≥10% was suggestive of better outcomes (but did not reach statistical significance probably due to small patient numbers in the subgroups) suggesting that factors in clonal biology other than just secretion of light chains influence clonal outcomes which reflect in patient survival. There was a significantly lower number of patients with cardiac involvement within the ≥10% 'normal' PCs group (only eight patients). Five year survival for those with cardiac involvement and < and ≥10% normal PCs were 31% and 49% respectively. Interestingly, the outcome between the cardiac and non-cardiac patients was not significantly different when patients had ≥10% 'normal' PC on MFC. The reason for this is unclear. One possible explanation is the composition of the light chains in such cases. The serum free light chain assay measures all light chains (both the normal polyclonal and abnormal monoclonal light chains). This is critically important since it is only the monoclonal light chain component that will deposit as amyloid deposits and the polyclonal light chains may well interfere with the amyloid formation. This phenomenon is well recognised in patients with hereditary types of amyloidosis – when patients with hereditary transthyretin amyloidosis get cardiac involvement and are treated with a liver

transplant as a curative procedure, the mixture of TTR in their blood changes from both mutant and wild type ATTR (each produced by the mutant and wild type TTR alleles respectively) to only wild type ATTR. These patients then develop accelerated cardiac amyloidosis from rapid deposition of this wild type ATTR. Also patients who are homozygous for hereditary amyloidosis variants develop rapidly progressive disease compared to heterozygotes. This perhaps demonstrates a protective effect of 'normal' PCs despite cardiac involvement. The outcomes of both haematological responders and non-responders were superior when associated with ≥10% 'normal' PCs on MFC. The multivariate analysis confirms that outcome in AL patients at baseline ultimately dependent upon extent of cardiac involvement which dominates the clinical outcome and patients succumb to effects of organ damage. The nature of plasma cell clone does not influence early outcome. However, in landmark analysis, the PC clone appears significant as patients who had <10% normal PCs have poorer outcome along with those with advanced cardiac involvement and non-responders to treatment.

In summary, when outcome was assessed according to overall BM burden and MFC it was clear that the presence of ≥10% 'normal' PCs conferred a favourable outcome regardless of the BMPC burden. This study confirms the value of MFC in patients with AL amyloidosis. Abnormal PC populations are demonstrable in all patients confirming the utility of the assay for diagnostic purposes. This is particularly relevant for those patients with low BM burden. Similarly the presence / absence of 'normal' plasma cells by MFC had a significant effect on outcome which was demonstrable in patients with both AL-MGUS and AL-MM. There was a negative correlation between

the amount of ‘normal’ PCs and cardiac involvement. As a result perhaps, the proportion of ‘normal’ PCs could not uphold as significant factors on a multivariate model along with cardiac involvement. The exact mechanism by which the proportion of ‘normal’ PCs impact survival needs to be further explored but it appears that patients with low levels of ‘normal’ PCs are predisposed to develop cardiac amyloidosis, a well-established poor prognostic marker in the AL population. It is therefore recommended, that MFC be included in the diagnostic work up of all patients with AL. Further studies are required to determine how this additional prognostic data can be incorporated into existing prognostic models.

Chapter Eight: The prognostic role of Heavy and Light chain suppression in systemic AL amyloidosis

Introduction

A substantial proportion of patients (up to 30% in some series) succumb prematurely to disease related complications.[73] The value of current amyloidosis staging systems in assessing longer term prognosis of patients surviving past the initial few months remains unclear and appears to be limited.

Monoclonal intact immunoglobulins (M-Igs) are measurable only in about a quarter of patients for monitoring purposes.[83] The prognostic value of an intact M-Ig in systemic AL amyloidosis is unclear, with some series reporting poorer outcomes for patients expressing intact immunoglobulins.[211, 250] Recently available serum heavy/light chains (HLC) immunoassays not only allow quantification of Ig'κ and Ig'λ HLC from which Ig'κ / Ig'λ HLC ratios can be derived, giving an indication of clonality but also appear to be sensitive in identifying and quantifying levels of M-Ig in plasma cell dyscrasias.[251-254] The particular advantage of this assay over traditional methods of immunoglobulin measurements are that the former, for the first time, allows the quantification of the uninvolved (polyclonal) member of the pair as well as of the other immunoglobulin classes (e.g. in an IgGκ monoclonal protein expressing patient, levels of IgGλ, IgAκ, IgAλ, IgMκ and

IgMλ may be measured)– thus providing an accurate measurement of isotype and non-isotype specific immunoglobulin values as well as pair immunosuppression. The clinical and prognostic significance of immunoparesis in plasma cell dyscrasias remains a topic of ongoing debate. There are few studies evaluating the role of HLC suppression for prognostication in plasma cell dyscrasias. In myeloma, HLC suppression appears to predict for poorer outcomes.[255-257] This chapter describes the significance of immunoparesis as determined by HLC suppression in a population of newly diagnosed patients with systemic AL amyloidosis.

Methods

Patient selection

The study included unselected patients with AL amyloidosis seen at the National Amyloidosis Centre, with serum samples collected at the time of presentation, prior to any therapy, and stored at -80°C. A total of 170 patients with systemic AL amyloidosis were included. Patients fulfilling criteria for symptomatic myeloma were excluded. For survival studies cardiac involvement was defined in methods chapter and/or NT-proBNP ≥332ng/L.

Serum samples were tested for FLC concentrations (κ SFLC and λ SFLC), HLC concentrations (IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ) and total immunoglobulins (IgG, IgA and IgM) as described in the methods chapter. Immunoparesis was defined either by total immunoglobulin (Ig)

measurements as the concentration of any Ig class below the lower limit of normal (i.e. IgG<6g/L, IgA<0.8g/L, IgM<0.5g/L; total Ig suppression), or by HLC immunoassays as levels of any IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and/or IgMλ below the lower limit of their respective reference range (HLC suppression). Severe immunoparesis was defined as levels of two or more isotypes suppressed by ≥50% below the lower limit of normal.

Outcome measures and Statistics

Survival studies were performed on 163 patients with available follow-up data (median follow up 35 months (2.4 – 85.3 months). Differences in overall survival (OS) between patient groups were analysed using Kaplan-Meier survival curves with the log rank test used to indicate significance. The association of variables with OS was carried out with Cox proportional hazard model. A landmark analysis was carried out in patients surviving 6 months from study entry. *P* values were two-tailed with a significance level of 0.05. Statistical analyses were performed using SPSS 21 (IBM, Chicago, USA). Statistical differences for categorical values were calculated using the chi-square (χ^2) test. Survival graphs were generated using GraphPad/Prism 5 software.

Results

Baseline characteristics including demographics, clinical features and serum biomarkers for 170 AL amyloidosis patients are presented in Table 8.1. HLC measurements identified immunosuppression in 145/170 (85%) patients

(Suppression of HLC IgG isotypes in 118(70%), IgA and IgM in 60 (35%) and 91 (54%)) (Table 8.2). 80 (47%) had ≥2 HLC isotype immunoparesis. Severe immunoparesis was identified in 29/170 (17%) patients (Table 8.2). None of the patients had symptomatic myeloma but 21% had greater than 10% bone marrow plasma cell infiltration.

Table 8.1 Patients characteristics (n=170)

	Median (range) or n/N (%)
Age years	68 (34 – 85)
Age≥65	96/170 (56)
Male	104/170 (61)
Cardiac involvement	124/170 (73)
Kidney involvement	104/166 (63)
Liver involvement	45/165 (27)
PNS involvement	5/164 (3)
GI tract involvement	16/167 (10)
>1 organ involved	66/170 (39)
NT-proBNP (ng/L)	1894 (9 – 69999)
Creatinine (mmol/L)	96 (19-851)
24h proteinuria (g)	3.1 (0.1-104.0)
Albumin (g/L)	35 (12-52)
Alkaline phosphatase (IU/L)	103 (27-3891)
Abnormal κ/λ SFLC ratio	134/170 (79)
kappa patients	48/134 (36)
lambda patients	86/134 (64)
dFLC ≥180mg/L	83/134 (62)
dFLC (mg/L)	237.9 (9.9 – 5026.8)
Abnormal HLC ratio	110/170 (65)
Intact M-Ig (by IFE)	87/170 (51)

115/166 (69%) had immunoparesis detected by total Ig measurements (IgG in 73(44%), IgA in 48(29%) and IgM in 66(40%)), all of which were identified by HLC suppression (p<0.001). 56 (34%) had suppression of ≥2 immunoglobulins, of whom 18(11%) were severe (Table 8.2).

Table 8.2 Frequency of immunoparesis by method

Method	**n**	**≥1 Ig suppressed n (%)**	**≥2 Ig suppressed n (%)**	**≥2 Ig suppressed >50% n (%)**
HLC suppression	170	145 (85)	80 (47)	29 (17)
Total Ig suppression	166	115 (69)	56 (34)	18 (11)
		HLC (≥1 Ig suppressed)*		
		No	**Yes**	**Total**
Total Ig (≥1 Ig suppressed)*	**No**	25	26	51
	Yes	0	115	115
	Total	25	141	166

*p<0.001 (χ^2 test)

Survival

There were 108 deaths of which 8 were due to infection and 89 due to progressive amyloidosis or amyloidosis. Median survival was 26.2 months (14.8 months for those with cardiac involvement). Factors adversely affecting outcome on univariate analysis were cardiac involvement, abnormal NT-proBNP and dFLC≥180mg/L (Table 8.3).

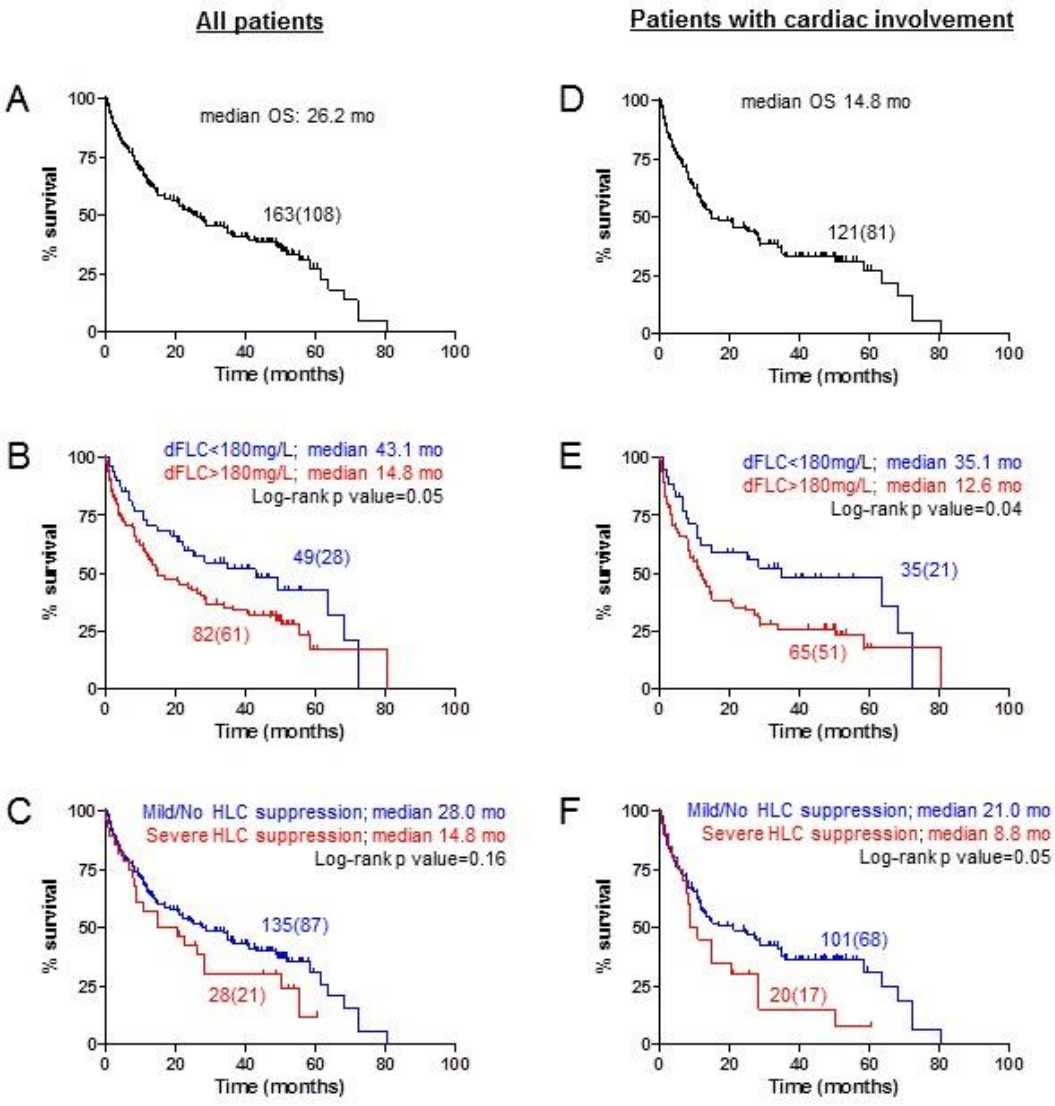
Table 8.3 Univariate analysis of risk factors for overall survival

	All patients						Patients with cardiac disease/ NT-proBNP≥332ng/L					
	OS (n=163)			6-month landmark OS (n=127)			OS (n=121)			6-month landmark OS (n=89)		
	n (%)	HR (CI)	p	n (%)	HR (CI)	p	n (%)	HR (CI)	p	n (%)	HR (CI)	p
Cardiac disease	61(37)	2.2 (1.5-3.4)	**<0.001**	40 (32)	1.8 (1.1-3.0)	**0.02**	-	-	-	-	-	-
NT-proBNP ≥332ng/L	115(71)	2.3 (1.3-4.2)	**0.006**	83 (65)	1.8 (0.9-3.6)	**0.08**	-	-	-	-	-	-
IFE positive	85(52)	1.1 (0.7-1.5)	0.81	70(55)	1.4 (0.9-2.3)	0.19	60(49)	1.1 (0.7-1.7)	0.67	45(50)	1.3 (0.7-2.3)	0.36
Abnormal FLC ratio	131(80)	1.2 (0.7-2.0)	0.49	98(77)	0.9 (0.5-1.5)	0.66	101(83)	1.2 (0.7-2.2)	0.49	72(80)	0.9 (0.5-1.9)	0.85
dFLC ≥180mg/L[1]	82(63)	1.6 (1.0-2.4)	**0.05**	88(69)	1.3 (0.8-2.3)	0.34	65(64)	1.7 (1.0-2.9)	**0.04**	43(60)	1.5 (0.8-2.8)	0.23
Abnormal HLC ratio	106(65)	0.9 (0.6-1.3)	0.48	74(58)	1.2 (0.7-2.0)	0.54	75(62)	0.9 (0.6-1.4)	0.7	59(66)	1.3 (0.7-2.4)	0.4
HLC suppression	140(86)	1.0 (0.6-1.7)	0.96	110(87)	0.9 (0.5-1.8)	0.77	104(85)	1.1 (0.6-2.1)	0.73	77(86)	1.1 (0.5-2.4)	0.83
HLC suppression (at least 2 Ig)	80(49)	1.0 (0.7-1.4)	0.92	64(50)	1.1 (0.7-1.7)	0.74	59(48)	1.0 (0.6-1.5)	0.85	45(50)	1.1 (0.6-1.9)	0.83
>50% HLC suppression (at least 2 Ig)	28(17)	1.4 (0.9-2.3)	0.16	22(17)	1.6 (1.0-2.9)	0.1	20(16)	1.7 (1.0-2.9)	**0.06**	15(17)	2.4 (1.2-4.6)	**0.009**
Total Ig suppression [2]	112(70)	1.1 (0.7-1.6)	0.8	87(70)	1.0 (0.6-1.6)	0.91	85(71)	1.2 (0.7-2.0)	0.44	63(72)	1.3 (0.7-2.4)	0.42
Total Ig suppression (at least 2 Ig)	54(34)	1.2 (0.8-1.8)	0.39	40(32)	1.1 (0.7-1.8)	0.77	45(38)	1.3 (0.8-2.0)	0.33	32(36)	1.2 (0.7-2.2)	0.48
>50% total Ig suppression (at least 2 Ig)	17(11)	0.9 (0.5-1.6)	0.65	14(11)	0.9 (0.4-1.9)	0.78	13(11)	0.9 (0.5-1.8)	0.81	10(11)	1.0 (0.4-2.3)	0.93

[1] Patients with abnormal FLC ratio only (n=134). [2] 4 patients missing data

Median OS for patients with dFLC levels above or below 180mg/L was 14.8 vs. 43.1 months, respectively (Hazard Ratio (HR) (95%CI): 1.6 (1.0-2.4); p=0.05), whereas median survival for patients with severe HLC suppression was 14.8 months compared to 28.0 months for all other patients (HR: 1.6 (1.0-2.9); p=0.09) (Figure 8.1a-c). Factors adversely impacting survival of patients with cardiac amyloidosis were dFLC ≥180mg/L and severe HLC suppression (Table 8.3). The median OS for dFLC ≥180mg/L vs. dFLC <180mg/L was 12.6 and 35.1 months, respectively (HR: 1.7 (1.0-2.9); p=0.04) and severe HLC suppression vs. without severe HLC suppression was 8.8 and 21 months respectively (HR: 1.7 (1.0-2.9); p=0.06) (Figure 8.1d-f) in this subgroup.

Figure 8.1a-f Survival outcomes in intention-to-treat (ITT) cohort. **a)** Median overall survival (OS) for the whole cohort (n=163); **b)** for patients stratified by baseline dFLC ≥180mg/L; **c)** severe HLC suppression (≥50% suppression in ≥2 Ig isotypes); **d)** Median OS for patients with cardiac involvement at diagnosis (n=121); **e)** stratified by baseline dFLC ≥180mg/L and **f)** severe HLC suppression. Number of patients (deaths) for each arm is shown.



The median OS for the 127 patients surviving ≥6 months at landmark analysis (Table 8.3 and Figure 8.2) was 40.9 months. Interestingly, dFLC ≥180mg/L did not have significant prognostic impact in this cohort (p=0.33), however, severe HLC suppression had a trend towards poorer survival (p=0.09). The latter was significantly associated with poorer outcome in patients with cardiac involvement within the 6 month landmark analysis, (HR: 2.4 (1.2-4.6); p=0.007). Similar observations were made at nine and twelve months landmark analysis (Figure 8.3).

Figure 8.2 Survival outcomes in 6-month landmark analysis, **a)** Median overall survival (OS) for all patients alive at six months (n=127); **b)** based on baseline dFLC ≥180mg/L; **c)** severe HLC suppression (≥50% suppression in ≥2 Ig isotypes); **d)** Median OS in the 6-month landmark analysis for patients with cardiac involvement (n=89); **e)** stratified by baseline dFLC ≥180mg/L and **f)** severe HLC suppression. Number of patients (deaths) for each arm is shown.

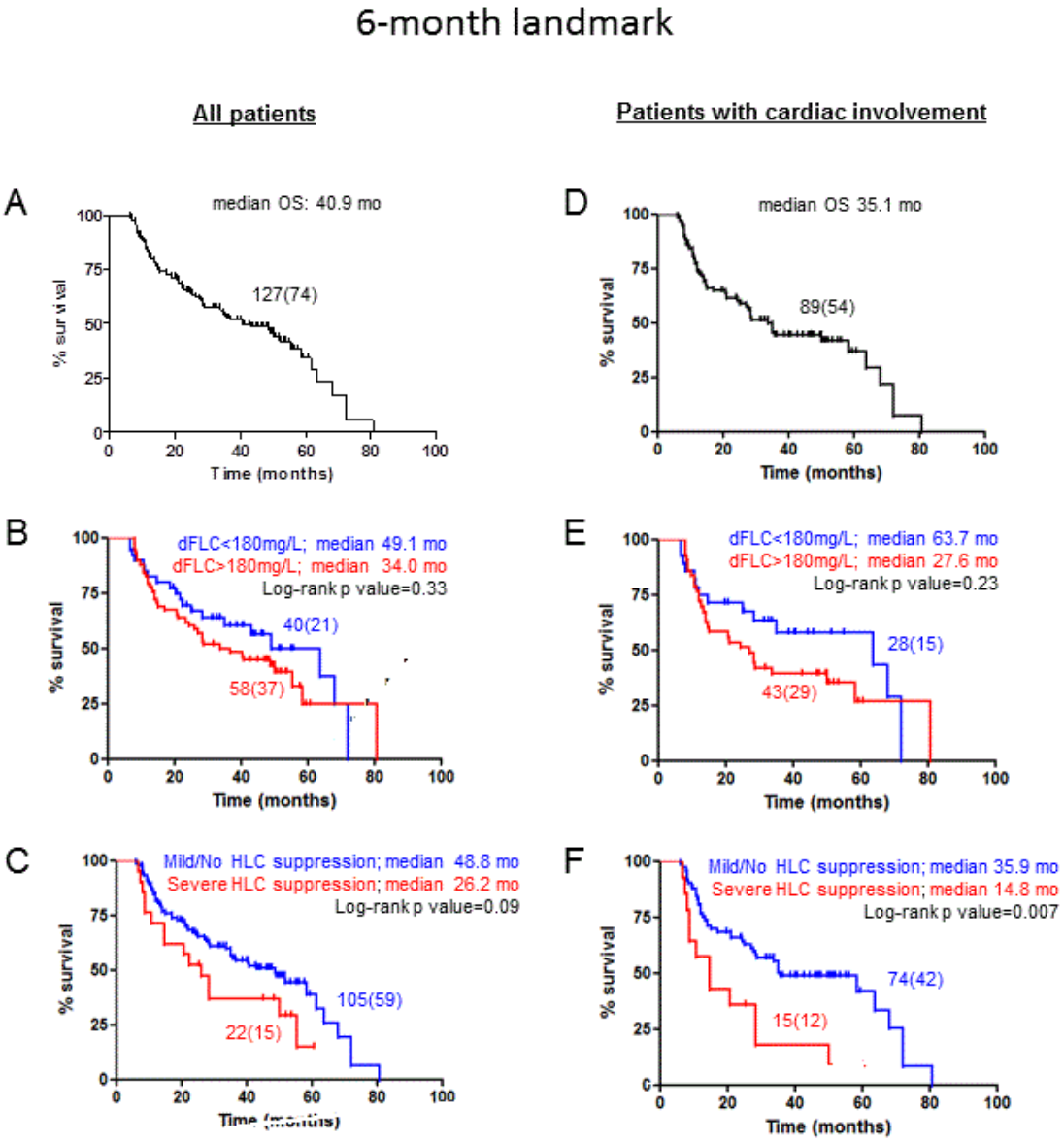
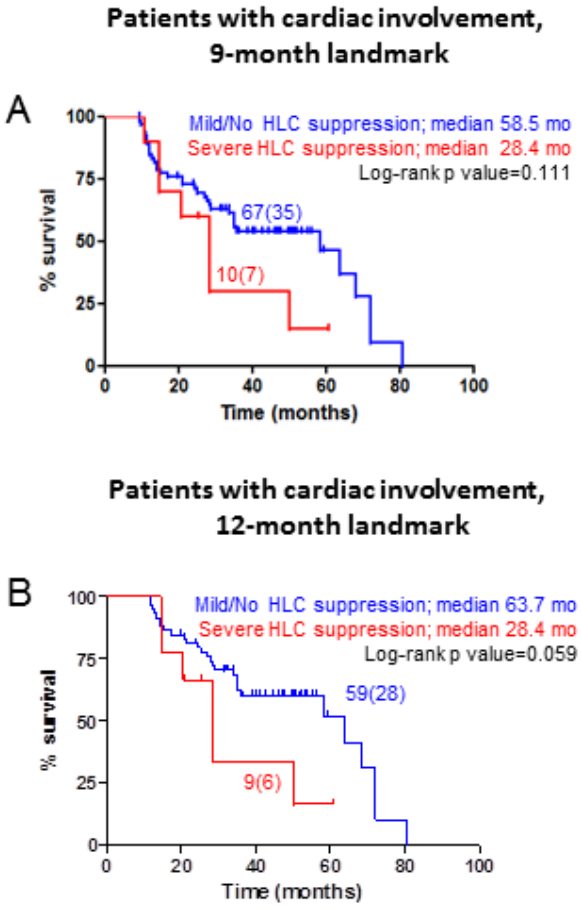
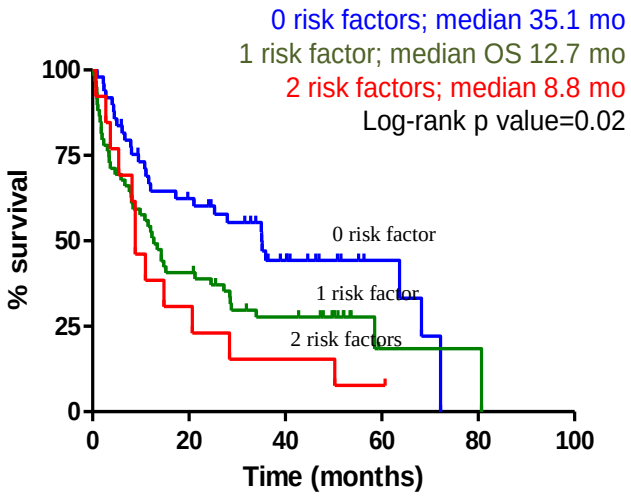


Figure 8.3 Survival outcomes at 9 and 12 month landmark analysis. Median OS in the **a)** 9-month (n=77) and **b)** 12-month (n=68) landmark analysis for patients with cardiac involvement and severe HLC suppression (≥50% suppression in ≥2 Ig isotypes). Number of patients (deaths) for each arm is shown.



A tentative survival model including dFLC ≥180mg/L and severe HLC suppression as risk factors in patients with cardiac involvement stratified the population into three categories with none (n=49), one (n=59) and two (n=13) risk factors and median survival times of 35.1, 12.7 and 8.8 months, respectively (p=0.023) (Figure 8.4).

Figure 8.4 OS survival for patients with cardiac involvement stratified by baseline risk factors (dFLC ≥180mg/L and severe HLC suppression). In 121 patients with cardiac involvement (cardiac disease and/or NT-proBNP ≥332ng/L) presence of none, one or two risk factors identified three groups with median survival times of 35.1, 12.7 and 8.8 months, respectively (p=0.02).



Discussion

The impact of immunoparesis on outcomes in plasma cell dyscrasias has been studied and debated for many years. This study specifically assesses immunoparesis and its impact in AL amyloidosis. Immunoparesis is common in systemic AL amyloidosis by both standard nephelometric immunoglobulin measurements and as determined by HLC immunoassays. Severe immunoparesis measured by HLC immunoassay, but not by total Ig measurement, is a marker of poor prognosis, particularly in patients with cardiac amyloidosis in a landmark analysis.

Impact of M-Ig on outcomes in AL amyloidosis is unclear. In this cohort the presence of an M-Ig by IFE had no prognostic value. Traditional electrophoretic methods lack sensitivity for detecting M-Ig and cannot accurately quantify the low levels typically encountered in patients with systemic AL amyloidosis.[83, 252] HLC immunoassays have a greater sensitivity for detection of M-Ig's and may aid the monitoring and prognostication of monoclonal gammopathies.[253, 256, 258]

Unlike myeloma, extreme HLC ratios are rarely seen in AL amyloidosis. By contrast, immunoparesis is nearly universal in myeloma and seen in a proportion of patients with monoclonal gammopathy of undetermined significance (MGUS).[256] An increased frequency of HLC suppression was also reported in a study in MGUS, in which 27% and 11% of 999 patients displayed immunoparesis as determined by HLC and total Ig measurements, respectively.[256] However systemic immunoparesis as determined by total Ig measurements remains an inconsistent risk factor both in MGUS and MM.[247, 257, 259-263] This cohort, demonstrated a greater

incidence of HLC suppression (85%) over total Ig immunoparesis (69%) in AL amyloidosis. Differences may partly be due to the ability of HLC immunoassays to separately identify kappa and lambda isotypes of each Ig class, unlike total Ig measurements. There was no correlation between immunoglobulin suppression and NT-proBNP or monoclonal FLC levels, indicating that polyclonal immunoglobulin levels do not associate with other risk factors or stage of disease in AL amyloidosis.[264]

Baseline level of dFLC was prognostic in this cohort as previously reported in other studies in AL amyloidosis.[130, 265] However, in the six months landmark analysis, the dFLC lacked prognostic power. The biggest challenge in AL amyloidosis is early deaths due to disease related complications. Patients surviving beyond six months have demonstrated resilience of organ function and have much better outcomes.[74] Baseline biomarkers don't have the same prognostic impact on the six month survivors.[266] The impact of baseline dFLC on survivors has (or indeed the lack of prognostic impact of baseline dFLC on survivors as seen here), to the best of our knowledge, never been previously reported. The striking observation in this series was the impact of severe immunoparesis on survivors in a landmark analysis. Patients with cardiac AL and severe HLC immunoparesis had a median survival of 8 months. By contrast immunoparesis by total Ig measurement (even severe immunoparesis), had no impact on prognosis in this study.

The mechanism of suppression of normal immunoglobulin components in plasma cell dyscrasias remains poorly understood but is likely to be directly related to the characteristics of the bone marrow plasma cell

clone. The group from Salamanca had reported that the presence of <5% normal plasma cells defined by multiparameter flow cytometry conferred a poor prognosis.[119] Similarly, as discussed in previous chapter, the presence of ≥10% normal PCs in the marrow by flow cytometry, irrespective of absolute PC percentage by morphology (also a prognostic factor as reported by the Mayo group), predicted for better outcomes.[267] Since normal immunoglobulin production is from persisting normal PCs in the bone marrow, the suppression of normal immunoglobulins as determined by HLC possibly represents the serum manifestation of this phenomenon.

Whilst this study shows the important prognostic impact of HLC immunoparesis on outcomes in AL amyloidosis, the reason for this prognostic impact is far more challenging to understand. Infections and worsening heart failure are the commonest causes of serious adverse events in patients with AL amyloidosis undergoing chemotherapy.[268] Drugs commonly used in treatment of AL amyloidosis such as dexamethasone or cyclophosphamide are excellent immunosuppressive agents, and are likely to eliminate normal plasma cells in addition to achieving the desired impact of clonal eradication. Since HLC immunoparesis of the "normal" uninvolved immunoglobulin is most likely to be directly linked to greater suppression/depletion of normal plasma cells, it is tempting to speculate that such patients with severe HLC immunoparesis will have worsening immunoparesis during treatment; which could tip these patients into a longer term state of immunodeficiency. The prognostic impact of HLC immunosuppression is greatest soon after completing therapy (i.e. in the six month landmark analysis) compared to patients alive at 9 or 12 months

suggesting, perhaps, that there may be immune recovery in the survivors; thereby mitigating the prognostic impact of immunoparesis. This immunoparesis may not only predispose to infective complication but may be a marker for poorer immune surveillance which may impact the longer term outcomes in plasma cell dyscrasia. An alternative, or even concurrent, reason may be that the suppression of normal plasma cells is a direct marker for the aggressiveness of the plasma cell clone as impacting on treatment responsiveness and possible persistence of minimal residual disease (MRD) with the attendant longer term consequences. We recently reported the persistence of MRD in AL amyloidosis patients in a serological CR,[269] highlighting the difficulty of eradicating even a small clone.

This study has limitations and these observations need to be validated in a larger patient population. The availability of baseline sera stored at the requisite temperatures dictated patient inclusion in this study. Baseline troponin measurement was not part of standard patient assessment at the UK NAC at time of this study and hence we are unable to present this data. The retrospective nature of the data limits the ability to assess cause of death and impact, if any, of infections due to immunoparesis; particularly worsened after chemotherapy. This study should be expanded to include HLC as part of baseline assessments in patients included in the ongoing observational study (ALCHemy) to validate these findings in a series of prospectively observed patient cohort. We hope that an ongoing serial study of HLC monitoring in AL patients during and after treatment may address some of these questions.

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

Chapter Nine: The role of bortezomib as front line treatment in patients with systemic AL amyloidosis

This chapter is written in the context of my publication:

A European collaborative study of cyclophosphamide, bortezomib, and dexamethasone in upfront treatment of systemic AL amyloidosis. Palladini G, Sachchithanantham S, Milani P, Gillmore J, Foli A, Lachmann H, Basset M, Hawkins P, Merlini G, Wechalekar AD. Blood. 2015 Jul 30;126(5):612-5. Copyright permission obtained from Blood office for use in my thesis.

Introduction

This final chapter will focus on the treatment of AL amyloidosis with particular attention to the currently, widely used first line, bortezomib based therapy and explore its effectiveness in patients within the different Mayo cardiac stages.

The introduction of bortezomib, the first-in-class proteasome inhibitor, represented a major advancement in the treatment of AL amyloidosis.[220, 270] Since the amyloidogenic clonal plasma cell is believed to rely on the proteasome to cope with the proteotoxicity caused by the misfolded light chain, bortezomib is expected to be particularly effective and a potential

targeted therapy in this disease.[152, 271] Early reports supported this expectation, showing a high response rate and rapid responses, particularly when this drug was used frontline.[153, 157, 158] A prospective clinical trial in relapsed/refractory patients showed that single-agent bortezomib, was rapidly effective, tolerable, and gave rise to durable responses.[155, 162, 272] After autologous stem cell transplant, bortezomib increases the rate and improves the quality of response.[175] Even more promising results were obtained when bortezomib was used frontline in combination with an alkylating agent and dexamethasone.[273] Moreover, two independent studies including a total of 30 patients receiving cyclophosphamide, bortezomib, and dexamethasone (CyBorD) frontline, reported a haematologic response in 90% of cases, with almost two thirds of patients reaching complete response (CR).[154, 274] This led to the perception that CyBorD was superior to other treatment alternatives, and this combination has become one of the regimens most commonly prescribed to patients with AL amyloidosis. However, this enthusiasm was soon tempered by the observation that CyBorD is not able to improve the outcome of patients with advanced cardiac involvement.[275] In a series of 60 patients with stage III cardiac AL amyloidosis the overall haematologic response rate was 68%, with CR in 17% of cases.[276] In this study, the overall median survival was almost one year, but patients who presented with NT-proBNP above 9500 ng/L had a median survival of only 4 months.[276] Moreover, two parallel matched case-control studies comparing bortezomib combinations with alkylating agents (CyBorD and BMDex) with the standards of care CTD and MDex, showed that the higher rates of good quality haematologic response obtained with

bortezomib-based regimens did not result in an improvement of overall survival.[225, 226] In these series, survival was driven by the high rate of early deaths in patients with advanced cardiac involvement identified by very high NT-proBNP concentrations and severe heart failure (>8500ng/L) who could not be rescued by bortezomib.[225, 226] However, a survival advantage was observed for lower-risk patients treated with BMDex.[226] These findings indicate that there is the need of large, collaborative studies to identify the patients who benefit most from these powerful combinations.[277] In this last results chapter, the outcome of 230 newly diagnosed patients with AL amyloidosis treated with CyBorD at two referral centres, the National Amyloidosis Centre (NAC, London, United Kingdom) and the Amyloidosis Research and Treatment Centre (ARTC, Pavia, Italy) are described .

Methods

Patient selection

The prospectively maintained databases of the ARTC and of the NAC were systematically searched for newly-diagnosed patients with AL amyloidosis treated with CyBorD between August 2006 and March 2013.

Outcome measures and Statistics

The primary outcome measures were the haematologic and cardiac responses. Secondary outcome measure was the time to next line therapy.

Data are presented as medians and interquartile ranges. Differences in response rates between subgroups were tested for significance by the χ^2 test or by Fisher's exact test as appropriate. Multiple logistic regression was used to compare response rates in various subgroups while adjusting for potential confounders. Response rates were reported by intent-to-treat. Survival curves were plotted according to Kaplan-Meier. Survival was calculated from the date of diagnosis to the date of last contact or death. Differences in survival were tested for significance by the log-rank test. Cox models were fitted to compute hazard ratios (HR) and 95% confidence intervals (CI) for survival. Multivariate models were fitted including non-collinear variables. The impact of response on survival was assessed in a three-month landmark analysis.

Results

A total of 230 patients (118 from the ARTC and 112 from the NAC), diagnosed between August 2006 and March 2013, were included in the study. Their clinical characteristics are reported in Table 9.1. Cyclophosphamide was administered at a dosage of 300mg/m^2 on days 1, 8, and 15 in all patients. The dosage of bortezomib ranged from 1.0mg/m^2 once weekly to 1.3mg/m^2 twice weekly. The maximum dosages of 1.6mg/m^2 weekly or 1.3mg/m^2 twice weekly were used in 60 patients (26%), and 79 subjects (34%) were treated with bortezomib 1.3mg/m^2 weekly. Bortezomib route of administration was intravenous in 154 patients (67%) and the remaining had subcutaneous injections. Most patients (184, 80%) received

at least 80mg dexamethasone per week. The median number of cycles performed was 4 (range 1-8 cycles).

Table 9.1 Characteristics of 230 patients with AL amyloidosis treated with cyclophosphamide, bortezomib and dexamethasone

Patients' characteristics	**N (%) or median (IQR)**
Male sex	134 (58)
Age, years	60 (53-66)
Organ involvement	
heart	169 (73)
kidney	157 (68)
soft tissues	35 (15)
liver	25 (11)
peripheral nervous system	6 (3)
Cardiac stage	
I	41 (18)
II	77 (33)
III	112 (49)
Stage III patients with NT-proBNP >8500 ng/L	45 (20)
NT-proBNP, ng/L	2839 (567-7018)
Renal stage	
I	115 (50)
II	90 (39)
III	17 (8)
dialysis	8 (3)
eGFR, mL/min per 1.73m^2	82 (61->90)
Proteinuria, g/24h	2.8 (0.4-6.9)
Bone marrow plasma cell infiltrate, %	12 (8-15)
dFLC (mg/L)	248 (96-567)
dFLC >180mg/L	135 (59)

IQR, interquartile range.

Treatment toxicity

Twenty-three patients experienced severe adverse events. The most common severe (grade 3-4) adverse event occurring during treatment was worsening heart failure (8 patients, 3%). Five of these patients were cardiac stage III, and the remaining were stage II. New York Heart Association (NYHA) class was III in 5 cases and class II in 3. Bortezomib was administered in 1.3mg/m^2 infusions in these patients, twice weekly in two cases and once weekly in six. All received dexamethasone at a weekly dose of 20mg. Two of these subjects are alive at 19 and 25 months, and the remaining died between 1 to 18 months. Other severe adverse events were hypotension (5 patients), renal failure (3), neuropathic pain (2), thrombocytopenia (2), lethargy (1), neutropenia (1) and psychosis (1). Additionally, 29 patients (13%) died within three months from diagnosis. Twenty-four of whom had Mayo stage III biomarkers, and NT-proBNP was >8500ng/L in 18 subjects.

Response to therapy

A total of 201 patients had measurable clonal disease, including 40 subjects who died before evaluation of response. By intent-to-treat, haematologic response was achieved in 138 of 230 patients (60%), with CR in 54 cases (23%). Of the evaluable patients, 125 (62%) reached haematologic response, that was CR in 42 cases (21%) and VGPR in 45 (22%). The response rate was significantly lower in cardiac stage III patients with NT-proBNP >8500ng/L (stage IIIb) (Table 9.2).

Table 9.2 Haematologic response rate by intent-to-treat according to cardiac stage in 201 patients with measurable disease.[221]

Response category	Stage I (30 patients)	Stage II (67 patients)	Stage IIIa (61 patients)	Stage IIIb (43 patients)
Overall response	23 (77%)	43 (64%)	42 (69%)	18 (42%)*
Complete response	10 (33%)	12 (18%)	14 (23%)	6 (14%)
Very good partial response	7 (23%)	18 (27%)	16 (26%)	4 (9%)
Partial response	6 (20%)	13 (19%)	12 (20%)	8 (19%)

*P<0.05 compared to stages I, II, and IIIa.

In a landmark analysis excluding patients who died within three months from diagnosis, 126 of 174 patients (72%) responded, with 42 CRs (24%) and 45 VGPRs (26%). Haematologic response rate was not significantly different in patients who received twice weekly and once weekly bortezomib (67% vs. 62%, P=0.549; ≥VGPR in 38% vs. 45%, P=0.446). However, patients who received less than 1.0mg/m^2 twice weekly or 1.3mg/m^2 once weekly bortezomib or less than 80mg per cycle of dexamethasone were less likely to achieve haematologic response (Table 9.3). In a multiple logistic regression analysis only cardiac stage IIIb (P=0.008), and not low doses of bortezomib (P=0.191) and dexamethasone (P=0.353), was an independent predictor of haematologic response.

Table 9.3 Haematologic response rate by intent-to-treat according to bortezomib and dexamethasone dosage in 201 patients with measurable disease.[221]

Response category	**Bortezomib dosage**		
	Full dose (35 patients)	**Intermediate dose (82 patients)**	**Low dose (79 patients)**
Overall response	29 (83%)	57 (69%)	42 (53%)*
Complete response	12 (34%)	20 (24%)	11 (14%)*
Very good partial response	7 (20%)	21 (26%)	17 (21%)
Partial response	10 (29%)	16 (19%)	14 (18%)
Response category	Dexamethasone dosage		
	Full dose (58 patients)	Intermediate dose (102 patients)	Low dose (41 patients)
Overall response	45 (78%)	62 (61%)	20 (49%)*
Complete response	15 (26%)	21 (21%)	6 (15%)*
Very good partial response	17 (29%)	23 (22%)	5 (12%)*
Partial response	12 (21%)	18 (18%)	9 (22%)

*P<0.05 compared to full dose

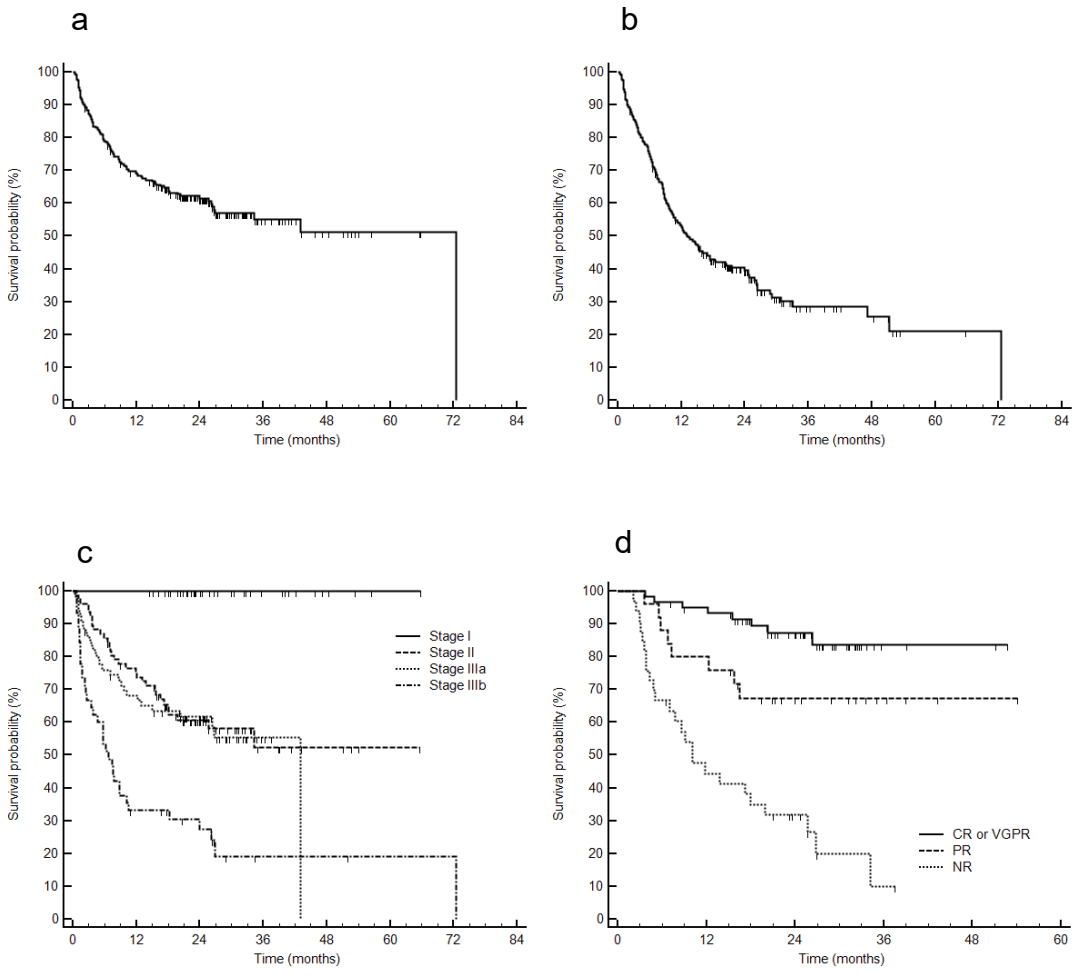
Bortezomib dosage: full dose, 1.3mg/m^2 twice weekly or 1.6mg/m^2 once weekly; intermediate dose, 1.0mg/m^2 twice weekly or 1.3mg/m^2 once weekly; low dose, less than 1.0mg/m^2 twice weekly or 1.3mg/m^2 once weekly. Dexamethasone dosage: full dose, at least 160mg per cycle; intermediate dose, <160 and ≥80mg per cycle; low dose, less than 80mg per cycle.

By intent-to-treat, 29 (17%) of the 167 patients with cardiac involvement achieved a cardiac response. Sixteen of 56 cardiac stage II (29%), 11 of 66 stage IIIa (17%, P=0.124 compared to stage II), and 2 out of 45 stage IIIb patients (4%) responded. Overall, 40 of the 157 patients (25%) with renal involvement achieved renal response, which was observed in 16 out of 59 evaluable renal stage I (27%), 21 of 81 in stage II (26%), and 3 of 17 stage III subjects (18%). The difference in renal response rate between renal stages was not statistically significant. Of the 25 patients with liver involvement 8 (32%) responded.

Survival

The median follow-up was 25 months. Overall, more than 50% of patients are projected to survive 5 years (Figure 9.1a). Median time to second-line therapy or death was 13 months (Figure 9.1b). Cardiac stage was a major determinant of patients' survival (Figure 9.1c): there were no deaths amongst stage I subjects, while the median survival of stage IIIb patients was only 7 months. Interestingly, there was no difference in outcome between stage II and stage IIIa subjects. In a 3-month landmark analysis, achievement of a haematologic response resulted in a significant survival advantage in stage II and IIIa patients (Figure 9.1d), as well as in stage IIIb subjects (Figure 9.2).

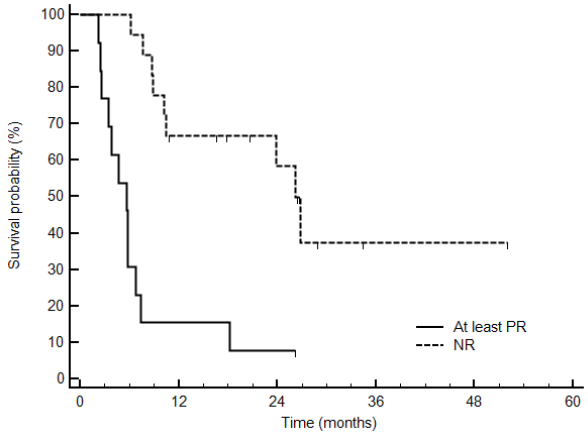
Figure 9.1a-d a) Overall survival of 230 patients with AL amyloidosis treated with CyBorD; **b)** Time to second-line therapy or death (median 13 months) of 230 patients with AL amyloidosis treated with CyBorD; **c)** Survival of 230 patients with AL amyloidosis treated with CyBorD according to cardiac stage; **d)** Survival of 118 cardiac stage II and IIIa patients according to haematologic response (3 month landmark analysis).[221]



Stage I, II, IIIa, and IIIb patients were 41, 77, 67, and 45, respectively. There were no deaths among stage I patients, and the median survival of stage II patients was not reached. Survival of stage II subjects was significantly shorter than that of stage I patients (P<0.001). The median survival of stage IIIa patients was 43 months, but their outcome was not significantly different from that of stage II subjects (P=0.613). The median survival of stage IIIb patients was 7 months (P<0.001 compared to stage IIIa).

Median survival was not reached for patients achieving at least PR, but subjects who obtained VGPR or better survived longer than those attaining PR (P=0.042). The median survival of non-responders was 10 months (P<0.001 compared to those in PR).

Figure 9.2 Survival of 31 cardiac stage IIIb patients according to haematologic response (3 month landmark analysis).[221]



The small number of patients did not allow discrimination between response categories. Median survival was 26 months for responders and 6 months for non-responders (P<0.001).

On a multivariate analysis, stage IIIb was the only variable retaining independent prognostic significance (Table 9.4). However, in the multivariable model based on the 3-month landmark, haematologic response also independently predicted the outcome (Table 9.4). Haematologic response had a major impact also on time to second-line therapy or death. In the overall population, median time to second-line therapy or death was 51 months in patients achieving at least VGPR, 13 months in patients attaining PR (P<0.001 compared to VGPR or CR), and 6 months in non-responders (P<0.001 compared to PR).

Table 9.4 Cox analysis of survival

Univariate analysis		
Variables	**HR (95% CI)**	**P**
Male sex	1.26 (0.83-1.91)	0.279
Age >60 years	1.21 (0.81-1.81)	0.361
eGFR <30 mL/min per 1.73 m^2	1.22 (0.63-2.34)	0.560
dFLC >180mg/L	1.99 (1.28-3.10)	0.002
BMPC >10%	1.85 (0.96-3.58)	0.069
NYHA class III or IV	3.18 (2.10-4.82)	<0.001
Stage IIIb	3.74 (2.45-5.71)	<0.001
Haematologic response*	0.17 (0.10-0.29)	<0.001
Multivariate model based on baseline variables		
dFLC >180mg/L	0.97 (0.47-2.08)	0.968
BMPC >10%	1.65 (0.80-3.41)	0.181
Stage IIIb	4.77 (2.38-9.59)	<0.001
Multivariate model including response (3-month landmark)		
dFLC >180mg/L	0.81 (0.33-1.99)	0.815
BMPC >10%	1.98 (0.78-5.05)	0.154
Stage IIIb	4.11 (1.74-9.71)	0.001
Haematologic response	0.25 (0.11-0.53)	<0.001

*Three-month landmark.

Stage IIIb patients are defined by NT-proBNP >8500 ng/L and cTnT >0.035 ng/mL or cTnI >0.1ng/mL.

Second line therapy

A total of 98 patients required second-line therapy. The combination of lenalidomide and dexamethasone was the most common rescue treatment, being used in 20 patients. Fourteen patients (70%), including 3 refractory to CyBorD, responded to lenalidomide, 2 achieved CR, and 5 VGPR.

Seventeen patients underwent second-line autologous stem cell transplantation (ASCT). Seven of whom were refractory to CyBorD, 2 had achieved a PR and 8 VGPR. Eleven patients (65%) responded to ASCT, with 8 CRs (47%) and 1 (6%) VGPR. Four of the nine patients with cardiac involvement achieved cardiac response before transplant. There was no transplant-related mortality.

Eleven relapsing patients received second-line bortezomib-based treatment. Four had relapsed after achieving at least VGPR with first line CyBorD, 2 had achieved PR and 2 were non-responders. In 3 patients VGPR was restored by second-line CyBorD. The remaining 8 patients had an immune modulatory drug (thalidomide in 6 subjects and lenalidomide in 2) added to bortezomib and dexamethasone, and 7 of whom responded (2 CRs, 3 VGPRs, and 2 PRs).

Fifteen patients received different combinations including pomalidomide, thalidomide, bendamustine, and MDex. In the remaining patients second-line therapy was deemed necessary but had not been commenced at the time of analysis.

Discussion

The regimens that are more widely used in AL amyloidosis, such as autologous stem cell transplant and MDex, tend to share a common fate: after early enthusiastic reports of high activity in small series,[135, 278] other studies, in different settings with higher proportion of patients with advanced disease, had worse, quite disappointing results.[85, 136] Controlled studies, which are difficult to conduct in AL amyloidosis, or large series of unselected patients are required to establish the ideal setting for each treatment approach. This has eventually been done with stem cell transplant[223] and MDex,[222] which emphasize the need for a risk-adapted approach to the treatment of AL amyloidosis.

In this study of unselected subjects, overall response rates, particularly organ response, were lower than previously reported, and comparable to that observed with other regimens, such as MDex,[222, 226] CTD.[142, 225] This was due to the inability of CyBorD to reduce early mortality in high-risk (stage IIIb) patients. However, the CyBorD combination proved extremely effective in patients without heart involvement (stage I). In this group, 56% of patients achieved at least VGPR, with no mortality recorded, indicating that these subjects can achieve prolonged survival if they are treated frontline with a safe regimen that is able to induce deep responses and closely followed to promptly treat any relapse. In patients with potentially reversible heart involvement (stage II and stage IIIa), CyBorD was also very effective, with almost 50% of subjects reaching VGPR or better. Importantly, there was no difference in the survival between stage II and

stage IIIa subjects (the latter representing almost two thirds of stage III patients), identifying an “intermediate-risk” group comprising the “old” stage II and stage IIIa patients and indicating the impact of treatment regimens in redefining staging systems. Amongst patients treated with CyBorD, the major determinant of survival was the presence of very advanced cardiac dysfunction at diagnosis, defined as cardiac stage IIIb, with very high (>8500 ng/L) NT-proBNP. In a recent study, the median survival of 62 stage IIIb patients treated with risk-adapted MDex was 7 months.[222] In the present series there was no improvement in survival in this group of patients (median 7 months). This is in agreement with the observation that the addition of bortezomib to MDex does not improve the outcome of patients with NT-proBNP >8500 ng/L.[226] However, in the present study, stage IIIb subjects who survived at least three months from diagnosis had a significant improvement in survival if they responded to CyBorD (median exceeding 2 years). In the multivariate analysis, stage IIIb and haematologic response were independent determinants of prognosis. This supports the case for haematologic response to extend survival by preventing further worsening of cardiac damage. Timing of cardiac responses in stage IIIb remains unclear (low in our series at an early assessment time point) and may well be delayed in this advanced setting. This emphasizes the importance of striving for a good and rapid response even in this poor-risk group.

In the present study CyBorD was well tolerated. However, despite rigorous prospectively maintained databases, the retrospective nature of this study might result in underestimating treatment toxicity. Close monitoring and careful supportive therapy of patients with NYHA class ≥II is warranted

during treatment with CyBorD, and cardiac toxicity may be managed by reduction of the dose of bortezomib.

The present study also allowed some observation on second-line therapy after CyBorD. Transplant eligible candidates who fail to achieve CR with frontline CyBorD can be transplanted and to improve the quality of their haematologic response. Also, patients who attain cardiac response but not CR with CyBorD may become eligible for autologous stem cell transplantation and be transplanted safely. In agreement with a previous observation,[279] immune modulatory drugs, particularly lenalidomide, are effective rescue agents after CyBorD, and can be combined with dexamethasone alone or added to a bortezomib-based regimen, granting a haematologic response.

In conclusion, Mayo stage I patients, without cardiac involvement, seem to benefit most from CyBorD and can be considered for autologous stem cell transplant if they fail to achieve CR. For patients with potentially reversible cardiac involvement (stages II and IIIa) who cannot be enrolled in clinical trials, CyBorD is a useful, highly effective upfront option. This study suggests, bortezomib combinations are not superior to the standard regimens in patients with advanced cardiac disease (stage IIIb). Nevertheless, these subjects should still receive chemotherapy with close monitoring and supportive care, since response can result in substantially improved survival in a minority of patients.

Chapter 10: General Conclusions

The studies in this thesis reveal a number of novel findings relating to the phenotype, investigations, prognosis and management of AL amyloidosis.

The proportion of elderly patients seen at the National Amyloidosis Centre is steadily increasing, mirroring general longevity and improved awareness of AL amyloidosis. The overall presenting features of the elderly patients with systemic AL amyloidosis are comparable to that of the general AL population. Perhaps due to multiple co-morbidities obscuring timely accurate diagnosis, a higher proportion of patients present with advanced stage disease. These two factors together means that the treatment of these frail elderly patients is challenging. Often both patients and clinicians may choose to avoid treatment altogether in view of the potential treatment related toxicity. This very first study focusing specifically on AL amyloidosis among older patients supports the treatment of cautiously selected patients. Clinical outcome is affected by patient selection as much as the specifics of therapy. As patients may not remain fit for salvage therapies, the first line treatment needs to be carefully chosen and should be highly effective with minimal toxicity profile. Patients without cardiac involvement and with good performance status are most likely to benefit. As previous studies have shown, a deep clonal response translates into better outcome and this holds true even in the elderly population as those with a VGPR or better had an excellent survival and organ responses.

IgD and IgM-related AL amyloidoses are rare subgroups of this condition with distinct clinical features and treatment outcomes and in need of better understanding. IgD AL patients have similar phenotype to the general AL population but the disease appears to have poorer long term prognosis, which, may be due to the relatively higher clonal burden and risk of progression to multiple myeloma which is unusual in AL amyloidosis. The condition is extremely rare and attention needs to be given to exclude IgD AL amyloidosis in those who appear to have an underlying light chain only secreting plasma cell dyscrasia.

The largest series of patients with IgM related AL amyloidosis reported here confirms that this is a distinct clinical entity. The key feature is that the underlying B cell clone is predominantly a Non-Hodgkin's lymphoma; consequently, perhaps, lymph node involvement is more frequent in this subgroup of patients. Although IgM-AL patients have less frequent cardiac involvement, compared to non-IgM AL population, the rate of deep haematological response are relatively low in the IgM patients. This maybe attributable to the higher frequency of neuropathic involvement precluding the use of effective novel therapeutic agents such as bortezomib and thalidomide, that can cause neurotoxicity. The scarcity of deep response may also be due to the use of anti-plasma cell agents in those with an underlying lymphoma. This study emphasises the need for individualised therapeutic approach taking into consideration, both the clinical features and the underlying clone being targeted in the current era of novel therapies. Deep clonal response remains the therapeutic aim. Notably, this study highlighted that dFLC is evaluable in only a small proportion of patients with

IgM but these patients have a significant IgM paraprotein, thus, haematological response should possibly be assessed using both the FLC and M-protein so to prevent patients mislabelled as non-responders. In addition to the widely used and accepted prognostic criteria, the Mayo staging system, liver and neuropathic involvements were found to determine survival of IgM-AL patients. Subsequently, a better risk model combining the cardiac biomarkers with liver involvement and presence of neuropathy is proposed and requires further validation.

One of the diagnostic challenges in amyloidosis patients in general is the lack of non-invasive diagnostic tools. This is particularly so in patients with amyloid deposits at rare sites such as lymph nodes, lungs, and breast. The study on ^{99m}Tc-DPD scintigraphy as a tool for identifying and monitoring extra-cardiac AL amyloid deposits is the first of its kind. Albeit the study utilises small numbers of patients, the outcome of this study gives scopes for the use of the radiolabelled bisphosphonate bone tracers as a non-invasive aid to the confirmation of soft tissue AL amyloid deposits. It may also be useful for serial imaging to monitor response to therapy and disease progression of soft tissue amyloidosis. This imaging modality is most likely to benefit patients with IgM-related AL amyloidosis who have a relatively high frequency of lymph node involvement; treatment response or progression in these sites can often become difficult - particularly in distinguishing amyloid from the underlying lymphoma in the majority of these patients.

Multicolour flow cytometry is valuable in patients with AL amyloidosis. It is useful in determining the proportion of normal and aberrant plasma cells

in a condition that is known to have a low tumour burden. The presence of normal plasma cells as determined by MFC has a favourable outcome despite the overall plasma cell burden estimated on bone marrow trephines. The exact mechanism for this positive outcome is not clear but it is noteworthy, that the proportion of normal plasma cells negatively correlated with the presence of cardiac involvement and dFLC – both are well known determinant of survival outcomes in AL patients. Therefore, it maybe that patients with low levels of normal plasma cells have a predisposition to developing cardiac amyloidosis. The outcome of the multicolour flow cytometry study provides an argument for including this technique in the regular diagnostic work-up of all patients with AL amyloidosis. This study did not find the overall bone marrow plasma cell burden determined on bone marrow trephine, to significantly affect survival.

Another possible prognostic marker arising from this thesis is the severity of immunoparesis as determined by heavy light chain measurement. This study showed that the majority of AL patients have some degree of immunoparesis that is not demonstrable by conventional methods. The study also highlighted that the prognostic markers at diagnosis do not necessarily govern the long term outcome in AL patients. Severe degrees of immunoparesis were associated with poorer outcome following treatment, in particular, severe immunoparesis significantly impacted the survival of patients with cardiac amyloidosis following treatment; suggesting that the aggressiveness of the plasma cell clone, determines the long term outcome in AL patients. Therefore, the measurement of degree of

immunosuppression by HLC method at presentation may help risk stratify patients and guide choice of therapy and management strategy.

The combination of cyclophosphamide, bortezomib and dexamethasone (CyBorD) is currently the most commonly prescribed regimens in the UK for AL amyloidosis patients. As a novel agent, bortezomib does provide high rates of hematologic response but this does not overcome the poor prognosis of advanced cardiac amyloidosis. The study on the use of bortezomib as first line therapy in AL patients confirmed the reasonably high levels of haematological response and subsequent much desired organ responses that are possible with this regimen. The study did however, highlight that it is those without cardiac involvement who most benefited from this regimen. There was also evidence that CyBorD can rescue subjects with reversible heart damage. Conversely, cardiac stage III patients with a high NT-proBNP had lower response rates translating into poorer median survival. Nevertheless, hematologic response also improved survival in these subjects, emphasizing the importance of striving for a good response even in those with advanced cardiac disease. The high clonal response and excellent outcome in early-stage AL amyloidosis with CyBorD confirm its place as a regimen of choice for this group.

Future studies

In the study of elderly patients, achieving a response with first line regimen was particularly important as outcomes for non-responders were similar to those not treated. Therefore, prospective trials with lower toxicity outpatient treatment regimens are needed. Prospective studies in older patients with novel agents with a better toxicity profile and ease of administration, such as oral proteasome inhibitors, may allow a greater proportion of patients to benefit from treatment.

As prospective studies are challenging due to the rarity of IgM-AL and IgD-related amyloidosis, international tissue and data registry would help to broaden the understanding of these rare subtypes. The revised staging system proposed in the IgM study requires further validation. Moreover, the roles of novel targeted therapies need to be further explored in this condition with predominantly underlying non-Hodgkin's lymphoma.

The findings of chapter six on the role of ^{99m}Tc-DPD scintigraphy requires further validation in a large group of patients with soft tissue amyloidosis, in particular, lymph nodes and amyloid deposits at sites such as the lung and breast which are rare. Moreover, the benefits of serial ^{99m}Tc-DPD scintigraphy also needs to be further explored in AL patients with soft tissue amyloidosis.

Further studies are required to explore the role of MFC and immunoparesis as determined by HLC, in existing prognostic models. The exact mechanism by which the proportion of 'normal' PCs impact survival

also needs to be further evaluated. The pathophysiological significance of the findings in the MFC study and the HLC study needs further exploration. Another study of particular interest would be the significance of minimal residual disease as determined by MFC and immunoparesis as determined by HLC method post-treatment.

Despite the availability of several novel agents and wider therapeutic options, there is a general lack of head-to-head evaluation of the treatments in AL amyloidosis and as such most of our knowledge comes from single-arm trials or retrospective studies such as that reported in chapter nine. Thus, large prospective studies are needed to identify patients who benefit most from novel agents such as bortezomib in order to guide management strategy.

Publications arising from this thesis

Imaging in systemic amyloidosis. Sachchithanantham S, Wechalekar AD. Br Med Bull. 2013;107:41-56. (Review article)

An evaluation of current treatment options for AL Amyloidosis. Sachchithanantham, S., & Wechalekar, A. D. and Hawkins, P. N. Expert opinion in orphan drugs. 2014:2 (3) pp. 229-244. (Review article)

Clinical profile and treatment outcome of older (>75 years) patients with systemic AL amyloidosis. Sachchithanantham S, Offer M, Venner C, Mahmood SA, Foard D, Rannigan L, Lane T, Gillmore JD, Lachmann HJ, Hawkins PN, Wechalekar AD. Haematologica. 2015 Nov;100(11):1469-76. (Original article)

European Collaborative Study Defining Clinical Profile Outcomes and Novel Prognostic Criteria in Monoclonal Immunoglobulin M-Related Light Chain Amyloidosis. Sachchithanantham S, Roussel M, Palladini G, Klersy C, Mahmood S, Venner CP, Gibbs S, Gillmore J, Lachmann H, Hawkins PN, Jaccard A, Merlini G, Wechalekar AD. J Clin Oncol. 2016 Jun 10;34(17):2037-45. (Original article)

Clinical profile and treatment outcomes of immunoglobulin D-Associated AL amyloidosis. Roussel M, Sachchithanantham S, Gibbs SD, Venner CP, Pinney JH, Gillmore JD, Lachmann HJ, Hawkins PN, Wechalekar AD. Br J Haematol. 2013 Sep;162(6):856-8. (Letter to editor)

A European collaborative study of cyclophosphamide, bortezomib, and dexamethasone in upfront treatment of systemic AL amyloidosis. Palladini G, Sachchithanantham S, Milani P, Gillmore J, Foli A, Lachmann H, Basset M, Hawkins P, Merlini G, Wechalekar AD. Blood. 2015 Jul 30;126(5):612-5. (Original article)

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