Amyloidosis: Incidence, Prognosis, Investigation and Management

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I, Jennifer Helen Pinney confirm that the work presented in this thesis is my own.

I have declared where information has been derived from other sources.

Abstract

Background:

Amyloidosis is a rare disorder of protein folding in which a variety of proteins deposit as fibrils in the extracellular space. The two most commonly affected organs are the kidneys and heart. Deposition of amyloid in these two critical organs is of major prognostic importance.

Aims:

To identify the burden of systemic amyloidosis in the English population. To characterise the phenotype and diagnostic features of wild type transthyretin amyloidosis and identify the type and frequency of arrhythmic activity in cardiac amyloidosis. To evaluate outcome in renal amyloidosis, and assess the role of renal replacement therapy.

Results and Conclusions:

Amyloidosis was mentioned in 0.58/1000 deaths in England between 2000 and 2008. Sensitivity of death certificates in identifying patients with amyloidosis was 79%. The estimated true incidence of the disease is ~1/100000 population in England.

Wild type transthyretin amyloidosis (ATTRwt) is increasingly diagnosed in the UK. Age of the patient at diagnosis and NT-proBNP level can aid in distinguishing ATTRwt from cardiac AL amyloidosis. Median survival is significantly better in ATTRwt than in cardiac AL amyloidosis. A positive troponin T, a pacemaker and NYHA class IV symptoms are all associated with worse outcome.

Complex ventricular arrhythmias are seen more frequently on Holter monitoring in patients with transthyretin cardiac amyloidosis compared to cardiac AL which are in turn more frequent than patients without myocardial amyloid infiltration. There does not appear to be an association between the frequency of complex ventricular arrhythmias and disease severity in cardiac amyloidosis.

Renal and overall outcome in AL amyloidosis are both strongly associated with FLC response and are best among patients who achieve >90% suppression of the monoclonal component of the FLC. Survival on dialysis is improving. Outcome following renal transplantation is dependent on the amyloid fibril type and suppression of the precursor protein.

Ethical Approval

All individuals whose data has been used in the clinical research studies described in this thesis gave explicit informed consent by signing a consent form whilst visiting the centre. The consent form was approved by the Royal Free Hospital Ethics Committee (REC Ref 06/Q0501/42). 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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Contents

Abbreviations

Systemic amyloid A amyloidosis	AA
Hereditary apolipoprotein AI amyloidosis	AApoAI
Hereditary apolipoprotein AII amyloidosis	AApoAII
Angiotensin converting enzyme	ACE
Amyloid enhancing factor	AEF
Atrial fibrillation	AF
Hereditary fibrinogen A α-chain amyloidosis	AFib
Gelsolin amyloidosis	AGel
Light chain amyloidosis	AL
Hereditary lysozyme amyloidosis	ALys
Alkaline phosphatase	ALP
Autologous stem cell transplantation	ASCT
Hereditary systemic transthyretin amyloidosis	ATTRm
Senile systemic amyloidosis	ATTRwt
Atrio-ventricular	AV
Bence Jones Proteins	BJP
Body Mass Index	BMI
Blood pressure	BP
β-2 microglobulin	β2M
Calcium	Ca
Chronic allograft nephropathy	CAN
Chronic obstructive pulmonary disease	COPD
Cerebrovascular accident	CVA
Confidence interval	CI

Chronic kidney disease	CKD
Combined liver kidney transplant	CLKT
Cardiac magnetic resonance imaging	CMR
Complete clonal response	CR
Creatinine	Creat
C-reactive protein	CRP
R-1-[6-[R-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid	CPHPC
Free light chain difference	dFLC
Deoxyribonucleic acid	DNA
99mTc-3, 3-diphosphono-1, 2-propanodicarboxylic acid	DPD
Dialysis related amyloidosis	DRA
Electrocardiogram	ECG
Eastern Co-operative Group	ECOG
Ethylenediaminetetraacetic acid	EDTA
Ejection Fraction	EF
Estimated glomerular filtration rate	eGFR
End stage renal failure	ESRF
Familial amyloid polyneuropathy	FAP
Free light chain	FLC
Familial Mediterranean fever	FMF
Glycosaminoglycans	GAGs
Gamma-glutamyl transpeptidase	GGT
Gastro-intestinal	GI
Haemoglobin	Hb

Hazard ratio	HR
Hydrogen peroxide	H_2O_2
Implantable cardioverter-defibrillator	ICD
International Classification of Diseases	ICD-9/10
Interleukin-1	IL-1
Interleukin-6	IL-6
Inter-quartile range	IQR
Iso-volumetric relaxation time	IVRT
Interventricular septal thickness in diastole	IVSd
Potassium	K
Late gadolinium enhancement	LGE
Left ventricular	LV
Left ventricular internal dimension in diastole	LVIDd
Left ventricular posterior wall thickness in diastole	LVPWd
Magnesium	Mg
Monoclonal gammopathy of undetermined significance	MGUS
Major histocompatibility complex	MHC
Myocardial infarction	MI
Mitral valve deceleration time	MVdecT
National Health Service	NHS
Number	N
UK National Amyloidosis Centre	NAC
No response	NR
Non sustained ventricular tachycardia	NSVT
N terminal pro brain natriuretic peptide	NTproBNP

New York Heart Association Classification	NYHA
Office of national statistics	ONS
Orthotopic liver transplantation	OLT
Polymerase chain reaction	PCR
Phosphate-buffered saline	PBS
Partial response	PR
Post transplant lymphoproliferative disorder	PTLD
Rheumatoid arthritis	RA
Renal replacement therapy	RRT
Renal transplant	RTx
Serum amyloid A protein	SAA
Serum amyloid P component	SAP
Standard deviation	SD
Strategic Health Authority	SHA
Tissue Doppler imaging	TDI
Tumour necrosis factor	TNF
Thyroid stimulating hormone	TSH
Transthyretin	TTR
University College London	UCL
Ventricular tachycardia	VT
World Health Organisation	WHO

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Chapter One: Introduction

Amyloidosis is a disorder of protein folding in which various proteins auto aggregate in a highly abnormal fibrillar conformation. Amyloid fibrils accumulate in the extracellular space, the deposits progressively disrupt the structure of tissues and this in turn affects organ function throughout the body.[1] Amyloid type is classified according to the fibril protein, and some 25 different proteins are known to form amyloid fibrils in vivo[2] (Table 1.1). Deposition of amyloid is diverse ranging from localised deposits that can be an incidental finding, to a progressive and sometimes rapidly fatal systemic disease. The anatomical distribution and natural history vary greatly between, and sometimes within, fibril types. Precise pathological diagnosis and comprehensive clinical evaluation are imperative for appropriate clinical management.

Fibril Formation and Amyloid Proteins

Fibrillogenesis of amyloid remains poorly understood. Experiments have shown *in vitro* that given specific conditions nearly any polypeptide chain can be driven towards misfolding and aggregation,[3] but, relatively few proteins are amyloidogenic *in vivo*. The polypeptides involved in amyloidosis are structurally diverse in their normal conformation and may be notably rich in β-sheet, α-helix or β-helix.[4] During amyloidogenesis, multimeric proteins dissociate to their monomeric components, and may further be enzymatically cleaved before or during their conversion into amyloid fibrils.[5,6] There are essentially three

circumstances in which amyloid deposition occurs. The first is a when there is sustained abnormally high concentration of 'normal' proteins that are usually present at low levels, such as serum amyloid A protein (SAA) in chronic inflammation, underlying susceptibility to AA amyloidosis. The second is when there is normal abundance of a 'normal', but to some extent inherently amyloidogenic protein over a very prolonged period, such as transthyretin in senile systemic amyloidosis (ATTRwt). The third situation is the presence of an abnormal protein with a markedly amyloidogenic structure, such as monoclonal immunoglobulin light chains in AL amyloidosis and genetic variants of transthyretin, apolipoprotein AI and fibrinogen Aα chain etc. in hereditary amyloidosis. Despite the heterogeneity of the various precursor proteins, the morphological structure and histochemical properties of all amyloid fibrils are remarkably similar. The core structure comprises anti-parallel β-strands of polypeptide chains lying perpendicular to the long axis of the fibril.[7] When visualised with an electron microscope, amyloid fibrils are characteristically straight, non-branching and ~7-10nm in diameter.[8] Amyloid deposits also contain the non-fibrillar normal plasma protein, serum amyloid P component (SAP),[9] which is bound in a reversible calcium dependent manner to a ligand present on all amyloid fibrils. Binding of SAP stabilizes the amyloid fibril and protects it from degradation by proteases and phagocytic cells in *vitro*.[10] Amyloid deposits also contain several other common constituents such as heparan sulphate and dermatan sulphate proteoglycans and glycosaminoglycans (GAGs), apolipoprotein E, type IV collagen and laminin. Glycan molecules may also contribute to the stabilization of the fibrillar conformation, and may also promote fibrillogenesis.[11]

Table 1.1 Classification of Systemic Amyloidosis by Precursor Protein

Type	Fibril Protein Precursor	Clinical Syndrome
AA	Serum amyloid A protein	Reactive systemic amyloidosis associated with chronic inflammatory diseases
AL	Monoclonal immunoglobulin light chains	Systemic amyloidosis associated with monoclonal plasma cell dyscrasias
AH	Monoclonal immunoglobulin heavy chains	Systemic amyloidosis associated with monoclonal plasma cell dyscrasias
Aβ_2M	β_2-microglobulin	Periarticular and, occasionally, systemic amyloidosis associated with long-term dialysis
ATTRwt	Normal plasma transthyretin	Senile systemic amyloidosis with prominent cardiac involvement
ATTRm	Genetically variant transthyretin	Familial amyloid polyneuropathy
ACys	Genetically variant cystatin C	Hereditary cerebral haemorrhage with cerebral and systemic amyloidosis
AGel	Genetically variant gelsolin	Predominant cranial nerve involvement with lattice corneal dystrophy
ALys	Genetically variant lysozyme	Non-neuropathic with prominent visceral involvement
AApoAI	Genetically variant apolipoprotein AI	Predominantly non-neuropathic with prominent viscera involvement
AApoAII	Genetically variant apolipoprotein AII	Non-neuropathic with prominent renal involvement
AFib	Genetically variant fibrinogen A alpha chain	Non-neuropathic with prominent renal involvement
ALect 2	Leukocyte chemotactic factor 2	Slowly progressive renal amyloid with nephrotic syndrome and liver involvement

Pathogenesis of Amyloidosis and Degradation

Although there is no doubt that substantial amyloid deposits disrupt organ function through their physical presence, it remains possible that pre-fibrillar amyloid aggregates may themselves have toxic effects. This hypothesis has so far mostly been explored in cardiac tissue.[12] The factors which determine the pattern of organ involvement is not fully understood. There can be major phenotypic

differences between members of the same kindred with the same genetic mutation that encodes for a particular variant protein in hereditary forms of amyloidosis. One hypothesis is that once amyloid fibrils have begun to deposit a template is formed. The amyloid fibrils themselves act as the amyloid-enhancing factor (AEF). The continued supply of the precursor protein then deposits in an exponential manner onto the template. This theory is supported by the evidence that the development of AA Amyloidosis in mice is markedly accelerated when protein from other AA amyloid-laden mouse tissue is injected, in addition to an inflammatory stimulus.[13]

Amyloid deposits are dynamic, with a continuous supply of the precursor protein the deposition of amyloid builds up leading to tissue damage, organ dysfunction and eventually death.[14] However when the supply of precursor protein is suppressed deposits of amyloid can be seen to regress.[15, 16] This has lead to the notion of a continuous process of amyloid 'turnover'.[17] The mechanisms by which amyloid is cleared from the body is not fully explained, but there is emerging evidence that clearance may be antibody mediated. Anti AA antibodies have been detected in mice that completely clear amyloid deposits.[18] Anti-AA antibodies can be detected in humans with inflammatory diseases. Lower concentrations have been reported in patients with rheumatoid arthritis (RA) and AA amyloidosis compared to controls with RA alone.[19] Peripheral administration of amyloid-specific antibodies has been a successful approach to reduce amyloid load in Alzheimer's disease[20] and AL amyloidosis.[21] Degradation of amyloid is in part macrophage driven. Macrophage depletion with liposomal clodronate has been shown to slow amyloid regression.[22] Murine macrophages have been shown to completely degrade Aβ-amyloid fibrils *in vitro*.[23] Infiltration of amyloid

deposits by macrophages is followed by the formation of multinucleated giant cells, which surround and engulf the amyloid. Some patients are seen to have rapid clearance of amyloid on SAP scintigraphy when there is suppression of the precursor protein whereas other patients will have no regression despite complete suppression. It has been postulated that different phenotype and function of macrophages may have a role here.

Epidemiology

Amyloidosis is a rare condition; the incidence of the disease is not well described. Approximately 500 new cases are referred to the UK National Amyloidosis Centre (NAC) each year and it has been estimated that 0.5-1.0 deaths per 1000 in the UK are due to the most prevalent AL type.[24] Very little population-based data are available to estimate the incidence of the disease. Kyle *et al* attempted to determine the incidence of AL amyloidosis from centralized records in Olmstead County. A total of 21 patients were found to have the disease during the period of 1952-1992, the annual incidence rate was 8.9 per million person years.[25] From this data the authors estimated that approximately 2225 new AL cases per year should occur in the USA. A group from Boston estimated the incidence of AL amyloidosis using mortality data. The diagnosis of the disease was made based on information recorded on the death certificate which might not have been specific to the underlying type of amyloid. The estimated incidence was as 4.5/100000.[26] Since 1994 there have not been any further estimates of incidence of the disease. Both previous reports described an increase in the incidence in the last few years

indicating a possible increase in the incidence of the disease or perhaps an improvement in detection.

Systemic amyloidosis is likely to be under-diagnosed. One study found the presence of AL amyloid in 38% of screening biopsies performed in patients with multiple myeloma,[27] However organ dysfunction from AL amyloidosis has only been estimated to complicate 3-7% of cases.[28]

The prevalence of amyloid was estimated by the AMYPRO study published in 2008. This study looked at histological samples with amyloid staining from any tissue over a 2 year period in Eastern France. An estimated 14 cases per million person years were found to have amyloid, 66 patients were identified, 40 cases (60.6%) were diagnosed with ATTRwt, 13 (19.7%) had AL and 9 (13.6%) showed AA staining.[29] These histological findings contrast with the proportion of patients actually diagnosed with the clinical disease where by far the most common type is AL amyloidosis.

Within the western world AA amyloidosis is reportedly under diagnosed. An autopsy study of 369 patients with RA found that the prevalence of cases nearly doubled from 18% of cases known to have amyloid in life to 30% at post mortem. Only 56% of cases where amyloid was found on renal tissue at autopsy had been reported to have proteinuria before death. Some deposits of amyloid may have therefore been incidental and subclinical or some patients may have been missed.[30]

There have been several studies describing the incidence of AA amyloidosis secondary to specific underlying diseases. There is much variability between countries depending on the prevalence and treatment of inflammatory conditions. In developing countries where there is limited access to health care,

and chronic infectious diseases such as tuberculosis are common, it is likely that AA amyloidosis is very much under diagnosed. The incidence of AA amyloidosis is much lower in the United States than in Europe, although the reason for this remains unexplained.[31,32] Even in cases with the same underlying disease there is reported variability between countries. A study of patients with Familial Mediterranean Fever (FMF) from 14 countries by Touitou *et al* in 2007, diagnosed 260 out of 2482 patients with AA amyloidosis. The country of recruitment was the main risk factor for developing amyloid.[33] Whether this reflects ascertainment bias, management variation or genuine environmental or ethnic differences is unclear but the latter two factors seem much the most likely. There is a suggestion from previous work at the UK National Amyloidosis Centre (NAC) and from a histology series in the USA, that patients of African origin are relatively less likely to develop AA amyloidosis, whereas those of Mediterranean or Semitic origins seem over represented in the cohorts studied. Whether this reflects differences in predisposition to amyloidosis or to diseases which confer a risk of AA amyloidosis is unclear although there is data that RA is less common in patients of African origin.

The incidence of hereditary amyloidosis has been little studied and varies widely between countries. Familial amyloid polyneuroapthy (FAP) is rare in the UK. The most common mutation worldwide is ATTRV30M which is well described in Portugal where the incidence is reported to be 3.1×10^{-5} with a gene carrier frequency of 1/1000.[34] Disease frequency varies widely between countries and within kindreds. The estimated number of carriers in Sweden was 7500 in a total population of 500000.[35] The most common aetiology of FAP in the UK and Ireland is the T60A variant.[36] Between 3-4% of black individuals have the V122I

transthyretin variant, which is associated with a predominantly cardiac phenotype that is thought to be clinically indistinguishable from ATTRwt.[37]

Diagnosis and Assessment of Organ Function

Histology

Diagnosis of amyloidosis is often made late in the course of the disease, and frequently as an unexpected histologic finding when a failing organ is biopsied. Congo red staining of tissue yielding the characteristic apple green birefringence under crossed polarized light remains the gold standard for confirming the presence of amyloid. The pathognomonic optical effect is produced by alignment of the dye molecules along the fibrils. It is not a very sensitive test; sensitivity is dependent on several factors: the presence of an adequate amount of amyloid, use of sufficiently thick tissue sections, technically correct staining and visualization procedures, and adequate observer experience. Target organ biopsies are usually diagnostic; however random 'screening' biopsies of fat or gastro-intestinal (GI) tract may not be positive due to the patchy nature of the deposits. The sensitivity of rectal biopsies has been estimated to be between 75-94% in published series.[38] Fat pad biopsies are a safe and convenient screening tool but the yield can be low.[39] It is therefore very important to consider the affected organs when performing a biopsy as this may greatly increase the rate of detection.

After the Congo red stain confirms the presence of amyloid deposition, the protein composition of the amyloid fibril i.e. the type of amyloidosis, must then be ascertained, and this is most accessibly achieved by immunohistochemistry. Immunohistochemical staining of amyloid deposits can be confounded by many

factors including background staining and loss of antigenic determinants in the fibrillar conformation, this is especially true in the common AL type.

Proteomic analyses comprising mass spectrometry on amyloid material cut out from tissue sections by laser capture microscopy has lately been shown to be effective in a large proportion of cases.[40-42] Biopsy can be hazardous although this does appear to be organ specific. Significant bleeds have been reported in 5% of liver biopsies. This is attributable to the increased fragility of affected blood vessels and reduced elasticity of severely amyloidotic organs. Renal biopsies do appear less hazardous with no increased incidence of bleeds reported in a large series of patients with amyloidosis, monoclonal gammopathy of undermined significance (MGUS), or myeloma.[43]

Genetic Sequencing

It is estimated that between 5-10% of systemic amyloidosis is hereditary. Genetic testing is frequently required, but the results must be interpreted in combination with histological and clinical findings.[44-47] The clinical phenotype associated with particular mutations may vary and since penetrance is variable, patients with AL amyloidosis can occasionally have an incidental mutation.[48,49] Conversely, some patients with hereditary amyloidosis have a potentially misleading but coincidental MGUS.[49, 50]

Imaging

SAP Scintigraphy

Various imaging techniques can make an important contribution to diagnosis and evaluation of organ involvement in amyloidosis. Of these, only radio labelled SAP scintigraphy is specific.[51] SAP is highly concentrated within deposits accounting for up to 15% of the total mass. SAP is a calcium dependent ligand binding protein which binds to DNA and chromatin.[52] Following intravenous injection, ^{123}I-SAP rapidly equilibrates between the relatively small pool of endogenous SAP within the circulation and much larger pool of SAP within the extra vascular amyloid deposits. The radio labelled SAP reversibly binds to all types of amyloid and localizes in proportion to the quantity of amyloid present, enabling deposits to be visualised in a semi quantitative manner.[53] The dose of radioactivity is small; 80-90MBq for a six hour scan and 120-190MBq for a 24 hour scan, meaning that serial scans can be safely used. SAP scintigraphy can be used to visualise the anatomical distribution of amyloid deposits and when used over time is able to detect evidence of progression or regression of deposits within organs.[51] Radiolabelled SAP scintigraphy has routinely been used at the NAC since 1988.[51] Scans reliably demonstrate deposits in the liver, spleen, kidneys, adrenal glands and bones. Unfortunately there is insufficient resolution to identify deposits in hollow, diffuse or very small structures such as the GI tract, skin and nerves. It is also unable to reliably evaluate deposits in the heart and lungs due to movement and blood pool content.[54] Once patients have reached end stage renal failure (ESRF) uptake of the tracer into the kidneys lessens due to a reduction in the blood supply and deposits are often not visualized.

The extensive use of this technique has provided information on the distribution of deposits in different forms of amyloid. It is not always possible to obtain a suitable biopsy to prove the amyloid type histologically as some anatomic sites such as the spleen or adrenal glands are not readily accessible for biopsy. Scans have shown that the distribution of amyloid within an individual organ can be patchy which may explain cases of false negative biopsies. There is a surprisingly poor correlation between the quantity of amyloid present in a particular organ and the severity of organ dysfunction.[55]

Sometimes the distribution of uptake seen on SAP scintigraphy is pathognomonic of a particular fibril type. For example substantial bone uptake is virtually always diagnostic of AL amyloidosis[51] (Figure 1). This can be extremely helpful in patients who do not have biopsy proof of AL amyloidosis and may enable treatment to be initiated rapidly. Likewise in localized AL amyloidosis, the lack of visceral deposits is helpful in supporting the diagnosis which is important as systemic chemotherapy treatment would be inappropriate.[56]

In a study by Hazenberg *et al* the distribution of deposits in AA amyloidosis showed deposition in the spleen in 87% of the 54 cases reviewed. Signal was seen in the spleen and kidneys in 35%, spleen only in 23%, spleen, kidneys and adrenal glands in 20%, spleen, kidneys and liver in 8% and kidneys only in 3%. In AL amyloidosis the distribution of deposits was much more diverse, the most common abnormality was also the spleen, seen in 75% of cases. Bone marrow uptake was seen in 21% of cases. The diagnostic sensitivity of SAP scintigraphy in both AA and AL types is 90%.[57] SAP scintigraphy in hereditary systemic transthyretin amyloidosis (ATTRm) is often negative, however abnormal uptake has been seen in the spleen and kidneys, the frequency of which is dependent on

the underlying mutation. Renal deposits are seen on SAP scintigraphy in all patients with hereditary fibrinogen amyloidosis who have not yet reached ESRF, splenic uptake is seen in 89% and adrenal uptake in 21%[45,58] (Figure 1.1). Extensive liver and spleen deposition can be seen in hereditary apolipoprotein AI amyloidosis; renal uptake is also commonly seen, however, this is dependent on the underlying disease causing mutation. Few patients with hereditary lysozyme amyloidosis have had SAP scintigraphy performed, the only kindred reported demonstrated extensive deposits in the kidneys, spleen and liver with extremely slow progression of deposits noted over many years.[59]

SAP scintigraphy has demonstrated the dynamic nature of amyloid (Figure 1.2). It has become a useful tool in monitoring the response to treatment in patients and in exploring the variation in the degree of suppression of the precursor protein needed to stabilize disease. Unfortunately SAP scintigraphy has not been developed commercially and is limited to a few clinical centers in Europe. This is due to availability issues for human SAP and I^{123} and cost of labeling.

Figure 1.1 (Left) Anterior whole body scintigraphic image following intravenous injection of ^{123}I-human serum amyloid P in a patient with AL amyloidosis. Uptake is seen in the bones, a finding which is pathognomonic for AL amyloidosis, uptake is also seen in the liver and spleen. (Right) Posterior whole body SAP scintigraphic image in a patient with hereditary fibrinogen amyloidosis. Uptake is seen in the spleen and kidneys.

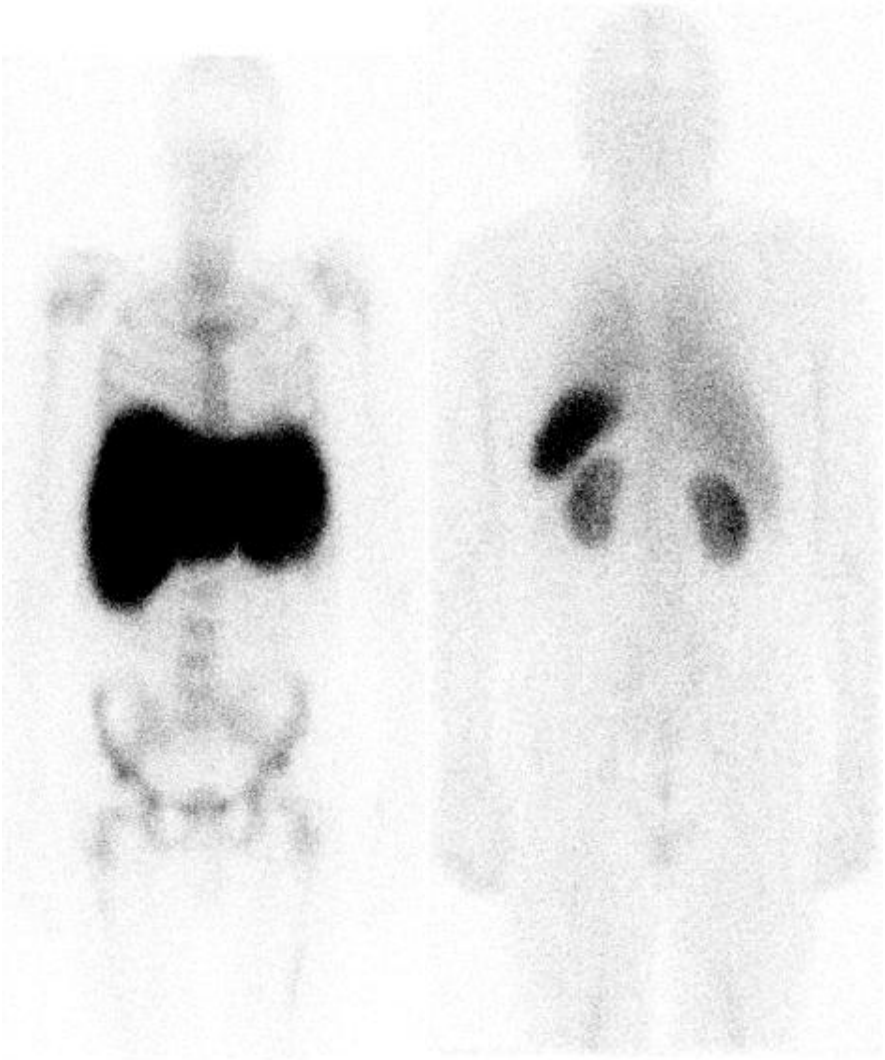
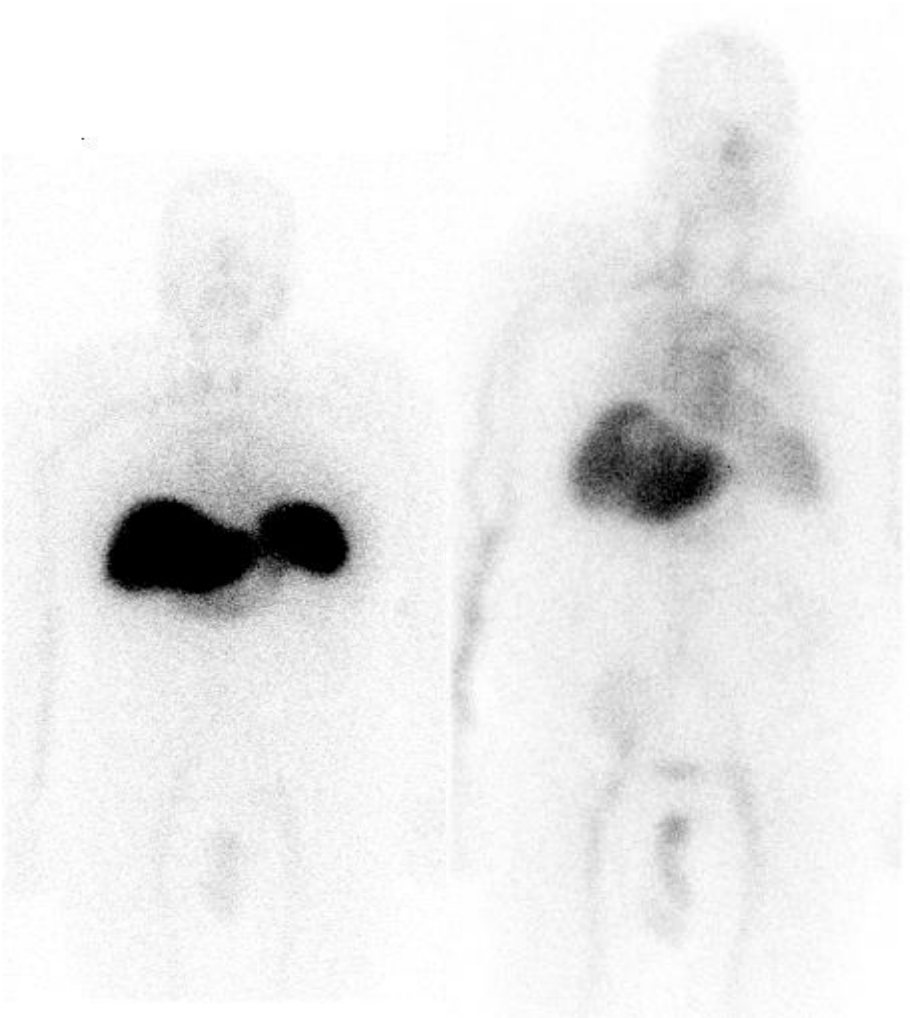


Figure 1.2 (Left) Anterior whole body SAP scintigraphic image in a patient with AA amyloidosis and end stage renal failure. Uptake is seen in the liver and spleen. (Right) The same patient with AA amyloidosis three years later demonstrating regression of amyloid deposits in the liver and spleen.



Cardiac Imaging

The degree of cardiac amyloidosis has a massive impact on prognosis in all types of systemic amyloidosis. There is much interest in utilising novel ways in which to characterise the degree of cardiac disease using a variety of imaging modalities. Echocardiography has long been used to demonstrate thickening of the ventricular walls and valves, and to evaluate the predominant diastolic restrictive abnormality that occurs in cardiac amyloidosis. Cardiac involvement in AL amyloidosis is defined as a mean left ventricular wall thickness of >12mm in the absence of hypertension or other causes of left ventricular hypertrophy.[60] Poor echocardiographic windows and inter-operator variability are significant limitations. Cardiac magnetic resonance imaging (CMR) has lately become more readily available and is now utilised in widespread clinical practice. This technique demonstrates very characteristic late gadolinium enhancement in sub-endocardium or in some cases more diffusely.[61,62] Availability of CMR throughout the UK has led to more frequent identification of cardiac amyloidosis and subsequent referral to the NAC from cardiology clinic. The role of CMR for monitoring progression or regression of amyloid has yet to be defined, the use of equilibrium CMR may prove a useful tool in quantification of amyloid, a technique which has been validated in fibrosis,[63] and recently in cardiac amyloidosis. This technique shows massive interstitial expansion in hearts with cardiac amyloid.[64]

99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) scintigraphy has more recently proved to be a useful tool in visualising cardiac amyloidosis.[65] DPD is a bone tracer used historically for bone scans. In patients with amyloidosis, tracer is seen to localise to the myocardium and muscles. The

degree of localisation and distribution appears to be different in patients with transthyretin amyloidosis compared to AL amyloidosis. This technique may be helpful in distinguishing between these two types of amyloid; the true sensitivity and specificity are yet to be ascertained in a large cohort of patients.[66] The degree of uptake is graded according to the Perugini scoring system: Score 0 - absent cardiac uptake and normal bone uptake. Score 1 - mild cardiac uptake, inferior to bone uptake. Score 2 - moderate cardiac uptake and normal bone uptake. Score 3 - Strong cardiac uptake with attenuated bone uptake.[67]

Cardiac Rhythm Analysis

The characteristic low QRS (limb leads <5mm) with poor R wave progression in the chest leads (pseudo infarct pattern) is the classical description associated with cardiac amyloidosis, and is reported to occur in up to 50% of patients with cardiac AL amyloidosis.[68] In practice a wide variety of electrocardiographic (ECG) changes and rhythm abnormalities are seen. In one series 21% had first degree atrioventricular (AV) block, 16% non specific intraventricular conduction delay and 3% second and third degree AV block. In the same series atrial fibrillation (AF) and atrial flutter was seen in 20% and 5% were reported to have non sustained ventricular tachycardia (NSVT).[69] ECG patterns alone cannot distinguish between types of amyloid but some patterns such as left bundle branch block are more commonly seen in ATTRwt.[70]

The characteristic ECG changes have historically been thought to be synonymous with cardiac involvement, such that the ECG has been incorporated into the diagnostic consensus criteria.[60] ECG findings have been shown not to have prognostic implications, and yet cardiac involvement is a strong prognostic

feature. Current literature has found that pacemakers or implantable defibrillators have not prevented sudden cardiac death resulting in the hypothesis that death is often due to electromechanical dissociation.[71] Defibrillator thresholds may be high due to the massive interstitial expansion of cardiac tissue.[72,73] Biventricular pacing appears to have a limited role, theoretically this may be the ideal pacing option to avoid decompensation of the stiffened ventricle as a result of induced dyssynchrony from right ventricular pacing.[74] There is limited literature describing the frequency and prognosis of rhythm disturbances in cardiac amyloidosis. Holter abnormalities have been shown to adversely affect survival. Complex ventricular arrhythmias are reportedly common but only couplets have been shown to be an independent predictor of survival and correlate with sudden death.[75]

Biochemical Analysis

Investigations for Clonal Disease

The source of the precursor protein in systemic AL amyloidosis is excess monoclonal light chains, produced by an underlying clonal disorder. All patients with amyloidosis should therefore have investigations performed to identify if there is an underlying clonal disorder. A plasma cell dyscrasia is identified in approximately 94% of patients with AL amyloidosis.[76] In patients seen as part of the ALchemy study at the NAC only one of 494 new patients with AL amyloidosis had no evidence of a plasma cell dyscrasia in either serum or urine tests.[77] Monoclonal proteins can be detected by serum and urine electrophoresis and immunofixation. The fully quantitative high sensitivity serum free light chain

immunoassay (Freelite) has improved the sensitivity of detection of an underlying clone.[78] Bone marrow examination and skeletal X-rays are required to exclude frank multiple myeloma. It is important to note that the incidence of MGUS occurs in at least 3% of people over 50 years old, and demonstration of a plasma cell dyscrasia therefore does not by itself confirm amyloidosis is of AL type. In fact, at the NAC 9.7% of patients with hereditary amyloidosis were initially wrongly diagnosed with AL amyloidosis based on the detection of an underlying clone.[44]

Serum Amyloid A Protein

Amyloid fibrils in AA amyloidosis are derived from the acute phase reactant SAA.[79] SAA is an apolipoprotein, which, like C-reactive protein (CRP) is synthesized by hepatocytes under the transcriptional regulation of pro-inflammatory cytokines, particularly tumor necrosis factor (TNF) alpha, interleukin-1 (IL-1) and interleukin-6 (IL-6).[80] The median plasma concentration of SAA in health is 3 mg/l,[81] but this can increase to over 2000 mg/l during an acute phase response. SAA has an inherent propensity to aggregate as amyloid fibrils and homozygosity for the $SAA_{1.3}$ allele has been proven to be a risk factor for AA amyloidosis.[82] The exact function of SAA remains unknown; there is speculation that its role may include modulating effects on reverse cholesterol transport and on lipid functions in the microenvironment of inflammatory foci. Under normal circumstances SAA is rapidly taken up by macrophages and transported to the lysosomal compartment and completely degraded. In patients with amyloidosis, intermediate SAA products appear to aggregate into fibrils. It is not known whether cleavage of SAA occurs before and/or after aggregation of

monomers during fibrillogenesis. After deposition of accumulated intermediates in the extracellular space, glycosaminoglycans, SAP and lipid components bind to the fibril and are thought to confer resistance to proteolysis.[10, 83, 84] The major determinant of whether amyloid deposits accumulate or regress is the SAA levels in an individual patient.[85] Remarkably minor changes in median SAA levels are associated with dramatic increases in the relative risk of death. In a time updated analysis patients who had an annualised median SAA of between 4-9mg/L were found to have a fourfold increase risk of death when compared to those with an SAA of less than 4mg/L (i.e. almost the same as healthy blood donors); the greater the median SAA level the higher the relative risk of death. Higher levels of SAA are also associated with a risk of progression to ESRF.[86] SAA levels are measured serially in order to identify the ongoing degree of inflammation in patients with AA amyloidosis, they act as a guide to whether the current treatment of the underlying disease is adequate.

Cardiac Biomarkers

The biomarkers N terminal pro brain natruiretic peptide (NT-Pro BNP) and cardiac troponins are also now widely used to provide information on cardiac involvement at diagnosis.[87] The MAYO staging system has prognostic implications for patients with AL amyloidosis. MAYO stage I is defined as NT-proBNP <332ng/L and Troponin T <0.035microg/L, MAYO stage II NT-proBNP >332ng/L and Troponin T <0.035microg/L, and MAYO stage III is defined by both NT-proBNP >332ng/L and Troponin T >0.035microg/L and confers the poorest prognosis.[88] It is not yet determined whether this staging system is applicable for use in patients with transthyretin amyloidosis.

Renal Biomarkers

Quantification of proteinuria is assessed at diagnosis and follow-up. Non Bence Jones proteinuria is commonly seen when there is renal involvement in systemic amyloidosis and, changes in proteinuria can indicate organ progression or response. The amount of proteinuria is extremely variable and can depend on the underlying fibril type. In AA amyloidosis the degree of renal impairment at presentation and serum albumin level have prognostic implications for both patient outcome and progression to ESRF.[86]

Liver Function Tests

The liver appears to tolerate amyloid deposition reasonably well. Organ dysfunction is frequently not seen despite large amounts of amyloid visualised on SAP scintigraphy. An elevated bilirubin is associated with a relative risk of death of 2.33 (95% CI 1.14 to 4.77, P = 0.02) in AL amyloidosis[89] and is felt to be a terminal sign when attributed solely to amyloid infiltration.[90]

General Management Principles

Excellent supportive care is vital for patients with all types of amyloidosis, in order to reduce the risk of complications and support organ function. This must be continued whilst awaiting amyloid regression in response to therapy directed at the underlying disease. Kidneys extensively infiltrated by amyloid are exquisitely vulnerable to inter-current insults such as hypo-perfusion, hypertension, nephrotoxic drugs and surgery, all of which should be avoided if possible.

Nephrotic syndrome is common in renal amyloidosis. General supportive management is therefore primarily directed at managing the, often, extensive oedema. In order to create a negative sodium balance patients are advised to limit their sodium intake to 3g per day and to restrict their fluid intake to less than 1.5L per day. Diuretics are often the mainstay of medical management, loop diuretics may be needed in high doses. A combination of loop diuretics with either thiazide, or potassium sparing diuretics can also be useful.[91] Angiotensin-converting enzyme (ACE) inhibitors have been shown to reduce proteinuria and reduce the risk of progression to ESRF in people with nephrotic syndrome.[92, 93] There is limited data on whether this extends to patients with amyloidosis. One study of 44 patients with AA amyloidosis did show a reduction of proteinuria in patients treated with Losartan (an angiotensin II receptor antagonist); whether this reduction in proteinuria confers any long term benefit in slowing renal progression in amyloidosis remains unknown.[94]

Patients with nephrotic syndrome are susceptible to infection; the last Cochrane review updated in 2009 concluded that there was no strong evidence for recommending any interventions to prevent infection in patients with nephrotic syndrome.[95] In AA amyloidosis due to chronic infection targeted management of

the disease is to prevent and treat infection in order to suppress SAA levels. Prophylactic antibiotics in other patients are not recommended but care must be taken to avoid infectious episodes if possible and patients are recommended to have vaccinations such as seasonal influenza, haemophilus influenza and pneumococcus.

Anticoagulation remains a controversial issue in nephrotic syndrome with no current consensus. Patients with amyloidosis have an increased risk of bleeding, due to fragile vessels and there are case reports of spontaneous splenic rupture.[96] Patients with very heavy proteinuria are at increased risk of thrombosis and the decision to anticoagulate must be made on an individual patient basis. General management principles apply to patients with amyloidosis for lipid lowering and diet as in other causes of nephrotic syndrome.

In AA amyloidosis adrenal involvement is common, but clinical adrenal insufficiency is rare. Often patients are on treatment with steroids for their underlying disease. Addisonian symptoms may be difficult to determine as hypotension can be due to reduced orthostatic pressure secondary to nephrotic syndrome, and diuretic use can lead to hyponatraemia. It is prudent to investigate patients for adrenal insufficiency if there are unexplained symptoms.[97]

Direct gastrointestinal tract infiltration can be seen in both AA and AL amyloidosis and chronic diarrhea can be a disabling symptom, often resistant to conventional therapies. Somatostatin analogs have provided relief in some case studies.[98, 99] Pseudo-obstruction can occur due to amyloid deposits in the myenteric plexus; in general promotility agents are not often successful. There has been some reported success with Cisapride, a prokinetic agent that acts by

releasing acetylcholine from the myenteric plexus,[100] but total parenteral nutrition may be needed to support the patient until bowel function improves.

Autonomic failure can be seen in AL amyloidosis and is particularly difficult to manage, especially in combination with cardiac involvement or nephrotic syndrome.

Management of heart failure secondary to cardiac amyloidosis is lacking in evidence. Patients with AL amyloidosis do not tolerate hypotension well and anti-hypertensive medications often need to be discontinued. There is currently no evidence to support or refute the use of anti-arrhythmic agents such as B-blockers or amiodarone in cardiac amyloidosis. Patients found to have rhythm disturbances secondary to amyloid are currently treated in line with general guidelines. It is recommended that patients found to have cardiac arrhythmias are anticoagulated, this is especially important in patients with AL amyloidosis who are at additional risk of thrombosis with some forms of chemotherapy, such as thalidomide and lenalidomide. When there is a history of excessive bruising or haemorrhage the risk of anticoagulation may however outweigh the potential benefits.

Amyloid infiltration can be painful depending on the site. Peripheral neuropathy can be a direct consequence of infiltration of amyloid in the peripheral nerves, which can occur in AL and some forms of hereditary amyloid. Amyloid causes an ascending motor sensory axonal peripheral neuropathy. This is length dependent and invariably symptoms start in the toes and progress upwards.[101] Some forms of chemotherapy used to treat AL amyloidosis can also cause a peripheral neuropathy, this is also length dependent hence tall patients have an increased risk of neuropathy. Analgesics such as gabapentin, pre-gabalin and amitriptyline may be helpful.[102]

Dialysis

Outcome on dialysis is reportedly improving. Survival on dialysis is superior in AA patients compared to AL.[103] Cardiac involvement in both AL and AA amyloidosis is associated with worse survival.[103, 104] Treatment with chemotherapy is associated with better outcomes, whilst older age and baseline creatinine are both associated with worse survival on dialysis in AL patients. The most common cause of death on dialysis is sepsis or sudden cardiac death.[104]

Organ Transplantation

Organ transplantation is controversial in amyloidosis for several reasons. In AL amyloidosis survival has historically been thought to be too poor for patients to benefit from an organ transplant such as a kidney. The other major concern is recurrent disease in the graft and subsequent graft loss. In AA amyloidosis infectious complications were reported in a recent French series.[105] Deaths following transplantation are reported as predominantly due to infection or cardiovascular disease. In multivariate analysis older age and recurrent amyloid are both associated with an increased risk of death.

In some hereditary forms of amyloidosis where the precursor protein is produced in the liver (e.g. hereditary transthyretin and fibrinogen amyloidosis), liver transplantation can be used as a form of gene therapy. The donor organ produces a wild type form of the amyloidotic precursor protein and theoretically no further deposition of amyloid should occur. Domino transplantation can be an option in ATTRm as the liver in patients with variant transthyretin does not have deposits of amyloid within it. It is likely to take decades for amyloid to build up in a recipient.

Types of Amyloid

Localised Amyloid

Localised amyloid deposition results from the local production of fibril precursor proteins.[106] Most deposits are found to be AL type, associated with foci of low grade monoclonal B cells which secrete monoclonal immunoglobulin light chains in the immediate vicinity.[107, 108] The respiratory tract, urogenital tract, skin and orbits, are the most frequent sites of deposition but all are rare.[109] There are case reports of amyloid affecting almost any site ranging from intracranial amyloidomas,[110] laryngeal and oropharynx amyloidomas,[111] to localised amyloid of the vagina.[112] Local resection of the 'amyloidoma' can sometimes be curative,[113] but amyloid deposits can recur within the same site or elsewhere within the same tissue. Amyloid deposits that appear initially to be localised can sometimes be a manifestation of systemic disease, it is therefore important to fully investigate patients in order to exclude systemic amyloidosis.[114] Once established that the amyloid deposit is a localised area, the management is dictated by the area involved and the degree of symptoms. Due to the extremely rare nature of the disease, management strategies have been somewhat experimental ranging from radiotherapy[115] to carbon dioxide laser ablation.[116] If the lesions are not causing symptoms then clinical surveillance may be all that is required.

Localised masses of amyloid can be found at insulin injection sites when repeated administration to the same area has occurred over many years.[117] This form of amyloid stains with antibodies to insulin and has been termed iatrogenic A-Ins type amyloid.

Systemic Amyloidosis

Four major acquired forms of systemic amyloidosis have so far been identified these comprise; systemic light chain amyloidosis (AL), systemic amyloid A amyloidosis (AA), dialysis related amyloidosis (DRA) and senile systemic amyloidosis (ATTRwt).

Systemic AL Amyloidosis

Fibrils in AL amyloidosis are formed from fragments of monoclonal immunoglobulin light chains consisting of all or part of the variable domain (V_L).[118] AL amyloidosis is a rare complication of monoclonal gammopathies and can occur in association with any form of monoclonal B cell dyscrasia.[76] The plasma cell proliferation fraction is usually similar to that of MGUS.[119] Symptomatic myeloma is unusual, approximately 15% of patients have multiple myeloma based on bone marrow biopsy findings.[76] Median age at presentation is 50-60 years and both sexes are equally affected.[76] Presentation is extremely variable as almost any organ can be affected except the brain.

Clinical suspicion of amyloidosis should be raised in any patient with unexplained nephropathy, cardiac failure, peripheral or autonomic failure, hepatomegaly or splenomegaly, or any unexplained multisystem disease. Approximately 50% of cases involve the kidneys presenting with proteinuria and frequently nephrotic syndrome. Renal histology remains the commonest tissue in which the disease is identified. Patients with AL amyloidosis can develop acquired factor X deficiency, underlying some reluctance to perform biopsies in patients with AL amyloidosis. Factor X assays are important in patients with abnormal clotting, and replacement with fresh frozen plasma prior to biopsy in

those who are deficient is recommended.[120] Cardiac involvement causing heart failure at presentation occurs in 15-30% of cases. Symptoms of cardiac involvement are those of congestive cardiac failure, most commonly with progressive breathlessness.[76,121] Postural hypotension is common in patients with cardiac involvement however this can also be a feature of autonomic nerve involvement or intravascular depletion due to nephrotic syndrome. Liver involvement is the predominant presenting feature in relatively few cases but is a common finding on post mortem studies with between 62-90% reported.[122] Hepatomegaly and elevated alkaline phosphatase (ALP) are the most frequent findings,[123] which does not always correlate with the amount of amyloid deposited. Soft tissue involvement such as macroglossia is pathognomonic for AL amyloidosis and not a feature in other forms of amyloid.

The current management of AL amyloidosis is to aim to suppress the production of the underlying B cell clone with chemotherapy and in turn halt the production of amyloidotic light chains. Evidence suggests that remission of the underlying clonal disease is associated with preservation of organ function and in some cases remission of amyloid deposits. Early identification and treatment is associated with improved survival.[78] All current therapies in AL amyloidosis have been derived from experience in multiple myeloma, but adverse effects are far more frequent and severe in AL due to the multisystem nature of the disease. It is vitally important that an individual assessment is made for each patient and treatment regimens are tailored accordingly. A variety of therapies have been employed ranging from high dose autologous stem cell transplantation (ASCT) to oral based regimens. The mortality associated with ASCT is significant. In the UK over 10 years treatment related mortality was 23%, this fell to 13% in the second

half of the decade.[124] Higher mortality is associated with the number of organs involved, cardiac involvement, age, performance status and serum albumin level.[125, 126]

It is also important to note that ASCT itself poses a risk of ESRF and in patients where preventing further renal decline is the goal of therapy this should be carefully considered. Conventional non transplant chemotherapy has been used for over 25 years in this disease and novel agents are constantly being trialled. Supportive management is imperative in this disease. Management of heart failure and nephrotic syndrome with diuretics is vital especially during treatment when side effects of agents such as dexamethasone and thalidomide can cause massive salt and water retention.

Outcome on dialysis was previously reported to be in the order of 12 months.[127] Historically there has been some reluctance to dialyse patients with AL amyloidosis due to the perceived poor outcome and difficulty with those who have cardiac or autonomic involvement. Transplantation in amyloidosis has also been contentious due to concerns that patients may not survive long enough to benefit from an organ transplant and that amyloid may recur within the graft. The UK experience following solid organ transplantation has in fact has shown that very few patients develop clinically significant graft amyloidosis. In a recent report twenty-two patients had received renal transplants with a 67% 5 year survival and no graft failures due to recurrent amyloid. Fourteen patients received cardiac transplants with a 45% 5 year survival. In 8 patients cardiac transplantation was performed to enable a subsequent stem cell transplant and the median survival in this group was 9.7 years.[128] It is important to appreciate that

these transplant recipients represent only 2% of all patients presenting with AL amyloidosis, i.e. a highly selected group.

The prognosis of untreated AL amyloidosis is very poor with a median survival of only 6-15 months and a 10 year survival rate of <5%.[76] The prognosis however is somewhat dependent on the organs involved with cardiac and autonomic nerve involvement conferring a particularly poor prognosis.[76,121] In 232 patients with cardiac involvement, patients with symptoms of congestive cardiac failure had shorter overall survival.[121] Echocardiographic features in patients with cardiac involvement, such as shortened deceleration time and increased early diastolic filling velocity to atrial filling ratio, have been shown to be predictors of death.[129]

Survival has significantly improved with the development of more therapeutic options. A recent report from the Mayo clinic showed improved survival rates over time with a most recent cohort from 2003-2006 having a 42% overall survival after 4 years.[130]

Response to treatment is best monitored using serial serum free light chains in the majority of patients. The degree of haematological response needed to result in the gradual net regression of amyloid varies from patient to patient, reflecting the differing capacity among individuals to clear their amyloid deposits. Studies have shown that achieving a complete clonal response (CR) in patients who have received ASCT have better survival outcomes,[131] and patients who achieve a greater than 50% response to treatment were shown to have superior survival outcomes regardless of the treatment they received.[78]

Systemic AA Amyloidosis

AA amyloidosis is a rare complication of chronic inflammatory conditions. The fibrils in AA amyloidosis are derived from SAA. Longstanding elevation of SAA is a prerequisite to development of AA amyloidosis but it is rare and its incidence varies throughout the world. The commonest predisposing conditions in the Western world are the chronic inflammatory arthopathies, which account for over 50% of cases. In the developing world reported cases are mainly associated with underlying infection. Patients with hereditary periodic fever syndromes are especially susceptible, perhaps due to the life-long nature of these inflammatory diseases, and this risk is substantially increased when there is a family history of AA amyloidosis. Other rare causes include Castleman's disease, vasculitis and neoplasias such as lymphoma and mesothelioma (Table 1.2).[86] Biopsy and post-mortem studies have suggested a prevalence of up to 3-6% in RA,[132,133] and 11-13% in FMF despite availability of colchicine treatment for the latter.[134] The factors that govern susceptibility to AA amyloidosis in the face of a high SAA concentration are yet to be determined. Polymorphisms in the gene encoding for SAA_1 isotype may contribute in part.[135]

AA amyloidosis predominantly affects the kidneys, with more than 95% of patients presenting with proteinuria, and around 10% of patients having already reached ESRF at diagnosis. Splenic involvement is evident on SAP scintigraphy almost without exception, and deposits commonly occur in the adrenal gland, liver and GI tract, although usually without associated organ dysfunction. Cardiac and neuropathic involvement is extremely rare.[86] Patients with persistent inflammation frequently develop progressive renal dysfunction and ESRF within

5-10 years. Almost 60% have nephrotic syndrome at presentation, which confers a high risk of infection.

Treatment will differ according to the nature of the underlying chronic inflammatory disorder, and there has lately been much progress with biologic therapies for RA etc, but progressive renal dysfunction remains common and ESRF occurs in up to a third of patients. Median survival on dialysis is in the order of 4-5 years, which is similar to that among age-matched non-diabetic patients. Renal transplantation has been performed in selected cases with reportedly excellent outcomes.

Patient outcome has gradually improved, with a reported median survival of 133 months.[86] Outcome is poorer in association with older age, lower serum albumin and ESRF at presentation. Serum SAA concentration has a powerful and modifiable influence on outcome. Complete suppression of inflammation (SAA concentration persistently <5mg/L) is frequently associated with regression of amyloid and preservation of renal function.

Table 1.2 Conditions Associated with AA Amyloidosis

Inflammatory Arthritis Adult Still's Disease Ankylosing Spondilitis Juvenile Idiopathic Arthritis Psoriatic Arthropathy Reiter's Syndrome Rheumatoid Arthritis Gout **Chronic Infections** Bronchiectasis Chronic Cutaneous Ulcers Chronic Pyelonephritis Leprosy Osteomyelitis Q Fever Subacute Bacterial Endocarditis Tuberculosis Whipples Disease **Immunodeficiency States** Common Variable Immunodeficiency Cyclic Neutropenia Hyperimmunoglobulin M Syndrome Hypogammaglobulinaemia Sex Linked Agammaglobulinaemia HIV/Aids **Other Conditions Predisposing To Chronic Infections** Cystic Fibrosis Epidermolysis Bullosa Injected Drug Abuse Jejuno-Ileal Bypass Kartagener's Syndrome Paraplegia Sickle Cell Anaemia	**Hereditary Periodic Fevers** Cryopyrin associated periodic fever syndrome (CAPS) Familial Mediterranean fever (FMF) Mevalonate Kinase Deficiency (MKD or HIDS) TNF receptor associated periodic syndrome (TRAPS) **Inflammatory Bowel Disease** Crohn's disease Ulcerative colitis **Neoplasia** Adenocarcinoma of the lung, gut, urogenital tract Basal cell carcinoma Carcinoid tumour Castleman's disease Gastrointestinal stromal tumour Hairy cell leukaemia Hepatic adenoma Hodgkin's disease Mesothelioma Renal cell carcinoma Sarcoma **Systemic Vasculitis** Behcet's disease Giant cell arteritis Polyarteritis nodosa Polymyalgia rheumatic Systemic lupus erythematosis Takayasu's arteritis **Other** Atrial myxoma Inflammatory abdominal aortic aneurism Retroperitoneal fibrosis SAPHO syndrome Sarciodosis Sinus histiocytosis with massive lymphadenopathy

Dialysis Related Amyloidosis

Dialysis related amyloidosis (DRA) is a complication of long-term dialysis following ESRF. The underlying fibril is due to β_2-microglobulin (β_2M) which is the light chain component of the major histocompatibility complex (MHC) class 1 molecule. It is synthesized in all cells that express MHC class 1 molecules.[136] β2M is cleared from the body by the kidney. It is freely filtered by the glomerulus and reabsorbed by the proximal tubular cells.[137] When patients develop ESRF, β_2M accumulates and the circulating concentration rises from normal levels (1-2mg/L) to ~50-70 mg/L. Most cases present clinically after 10 years on dialysis and this form of amyloid has a tropism for osseo-articular surfaces. Symptoms of DRA manifest as carpal tunnel syndrome, arthralgia, spondyloarthopathy, subchondral bone cysts and fractures. Whilst modern high flux dialysis techniques may have reduced the incidence of β_2M amyloidosis, renal transplantation remains the only effective treatment in reducing the symptoms of established disease.[138]

Senile Systemic Amyloidosis, Wild Type Transthyretin Amyloidosis

Wild type transthyretin amyloidosis (ATTRwt) also known as senile systemic or senile cardiac amyloidosis is a disease of the elderly which usually affects men. The fibril is composed of normal wild-type transthyretin.[139] Amyloid deposits of this type are found at autopsy in about 25% of patients over age 80, with predominant involvement of the heart.[140] Non-clinically significant deposits are frequently present in other sites including the lungs, gut, bladder and small arteries in many other tissues.[141] A relatively small proportion of patients with

wild-type transthyretin amyloid deposits present with clinical disease, and among those who do, most present with restrictive cardiomyopathy and congestive cardiac failure. Wild type transthyretin derived amyloid has been found in specimens following carpal tunnel release surgery,[142] which often precedes cardiac manifestations.[143] Echocardiography demonstrates a markedly thickened myocardium, which is typically greater than in AL amyloidosis. In a study of 18 patients with ATTRwt the mean interventricular septum thickness was 17.8mm in the ATTRwt group as compared to 14.3mm in the AL group (P=0.002).[144] Identification of ATTRwt has lately increased due to the advent of CMR imaging, but definitive diagnosis continues to rest on endomyocardial biopsy confirming amyloid of transthyretin type in conjunction with wild-type TTR gene sequence.[145] There is limited data regarding the natural history and outcome of the disease, with current series limited by small numbers of patients. Progression of heart failure appears to be slow with much better median survival than AL amyloidosis.[144] The mainstay of management is supportive care and symptomatic management of heart failure with fluid restriction, low salt diet and diuretics.

The Hereditary Systemic Amyloidoses

Hereditary amyloidosis is a group of diseases due to mutations in different specific proteins. All are autosomal dominant but penetrance is variable and there is often no family history. Age of onset, disease penetrance and phenotype vary widely between different mutations and even within kindreds, presenting a challenge for genetic counselling. Hereditary systemic amyloidosis can be divided into neuropathic and non-neuropathic forms. The former comprise FAP, usually caused by mutations in the transthyretin gene (ATTR) and gelsolin amyloidosis (AGel). Non-neuropathic forms include fibrinogen Aα-chain amyloidosis (AFib), apolipoprotein AI amyloidosis (AApoAI), apolipoprotein AII amyloidosis (AApoA11), and lysozyme amyloidosis (ALys).

Hereditary Transthyretin Amyloidosis (ATTRm, Familial Amyloid Polyneuropathy)

The most common type of hereditary amyloidosis worldwide is associated with mutations in the gene for transthyretin. There are some 100 point mutations associated with the clinical syndrome, which is typically characterized by progressive peripheral and autonomic neuropathy. Cardiac amyloidosis is frequent, as are varying degrees of amyloid deposition in the viscera, vitreous, gut, and occasionally the central nervous system.[146,147] Clinical presentation is typically from the third decade, though this varies dependent upon the specific mutation.[148,149]

Transthyretin is a transport protein that circulates in a tetrameric form. More than 95% of transthyretin is produced in the liver, with the remainder

produced in the choroid plexus and retina.[101,150] The most common transthyretin mutation involves the substitution of methionine for valine at position 30 (ATTRV30M). This usually presents with an ascending sensorimotor peripheral neuropathy, and unlike most other mutations, cardiac involvement is rare. The disease typically develops by age 30-40 years in the Portuguese focus, but about 20 years later in the Swedish one. The most common aetiology of FAP in the UK and Ireland is the T60A variant.[36] This usually presents after the age of 50 and often with autonomic symptoms, but cardiac amyloid is virtually always present at diagnosis.[151] 3-4% of black individuals have the V122I transthyretin variant, which is associated with a predominantly cardiac phenotype that is clinically indistinguishable from ATTRwt; it usually presents after age 60 and is not associated with neuropathy.[37]

TTR amyloidosis has become a particular focus for development of novel anti-amyloid therapies, both with a view to stabilizing soluble TTR in the blood and inhibiting its production through silencing RNA and anti-sense oligonucleotide approaches. Several strategies have already progressed to clinical trial. However, the only therapy that has been adopted into widespread clinical practice is orthotopic liver transplantation (OLT). This technique was introduced in 1990 on the basis that almost all mutant TTR is produced in the liver.[152, 153] Since this date over 700 transplants have been performed. The Familial Amyloid Polyneuropathy World Transplant Registry reported seven years of registry data in 2003, the most common mutation was ATTRV30M representing 83% of cases and 5 year survival was significantly better in this group when compared to patients with non-ATTRV30M mutations (79% vs. 56% respectively, $P<0.001$). Symptoms of peripheral neuropathy were reported to gradually improve in up to

50% of cases,[154] with better outcomes associated when transplantation is performed earlier on in the course of the clinical disease.[151] OLT remains controversial; cardiovascular death is higher in patients with FAP as compared to other indications for liver transplantation, and progression of cardiac amyloidosis can occur afterwards due to on-going deposition of wild-type TTR in this particular anatomical site.[155, 156] The indications, timing and outcome of OLT for FAP remains unclear. This is especially true in patients with non-ATTRV30M mutations who already have cardiac amyloid deposits.

Hereditary Gelsolin Amyloidosis (AGel)

Hereditary amyloidosis of the Finnish type, in which the amyloid fibrils are derived from cleavage fragments of variant gelsolin, was first described by the Finnish ophthalmologist Jouko Meretoja in 1969.[157] This disease has since been reported in various other countries but the largest population remains in Finland. Two variants have been found, G654A and G654T. The G654A type has been reported in Portugal, Japan and Iran, whereas patients with the G654T variant have been reported in Denmark, the Czech Republic and France.[158] Gelsolin is an actin-modulating protein that enhances migration of cells. Mutated gelsolin is unable to bind calcium ions and it is thought that this may render it more prone to proteolysis and subsequent fibril formation.[159-161]

Typical presentation is with corneal lattice dystrophy during early middle-age with subsequent development of a slowly progressive but very disabling and deforming cranial neuropathy.[162] Life expectancy is near normal. Substantial renal amyloid deposits are present at an early stage; interestingly there is usually no

associated clinical evidence of visceral involvement. Rare cases of homozygous mutations have been reported with a rapid decline and renal failure.[163]

Fibrinogen Aα-Chain Amyloidosis (AFib)

Hereditary fibrinogen amyloidosis (AFib) is the most common cause of hereditary renal amyloidosis in the UK, it was first characterized in a Peruvian kindred in 1993.[164] Disease penetrance is variable and a family history is very often absent. To date, nine amyloidogenic mutations in fibrinogen have been identified with the E526V variant being the most common. Presentation is universally with proteinuria and renal impairment and the diagnosis is therefore almost always made on renal biopsy. Histological findings show a characteristic picture of massive glomerular amyloid infiltration with almost complete obliteration of the normal architecture but little or no vascular or interstitial deposits. Hypertension is common. Extra-renal amyloid deposits are usually evident in the spleen and sometimes adrenal glands on radiolabelled ^{123}I-labelled SAP scintigraphy. Liver involvement is rare, but can in some cases, be clinically significant.[45] Cardiac involvement has been reported in a few non-E526V associated cases. Presentation typically occurs around age 60 years, with progression to ESRF within about 5 years from diagnosis. The absence of clinically significant extra-renal disease is consistent with median survival from presentation of 15 years.[45]

Median graft survival following isolated renal transplantation is approximately seven years, recurrent amyloid disease is the main cause of graft loss beyond this stage. On the basis that the amyloidogenic protein is produced exclusively by the liver, combined liver and renal transplantation has been offered to some younger patients who have developed ESRF. Whilst further amyloid

deposition is prevented by these means, and long term outcome can be excellent, there has been substantial early mortality. Three out of nine reported cases died due to complications of surgery.[165]

Hereditary Apolipoprotein AI Amyloidosis (AApoAI)

Apolipoprotein AI is produced in the liver and intestines, and is catabolised in the liver and kidneys. It is the predominant protein in high density lipoprotein[166] and plays a key role in reverse cholesterol transport.[167] Whilst tiny wild-type apolipoprotein AI-derived (AApoAI) amyloid deposits have been identified in atherosclerotic plaques, some 13 variants have been associated with major visceral amyloidosis, i.e. hereditary systemic amyloidosis. The pathogenesis involves proteolytic cleavage, with the amino terminal 83-93 residues typically being incorporated into the amyloid fibrils.[168] Currently, of the more than 50 ApoAI variants known, 13 are associated with amyloidosis.[169] Different mutations are associated with a variety of phenotypes and again there is marked variability even within families which makes genetic counselling extremely challenging. The most common manifestation is renal involvement with subsequent chronic renal failure but there may also be significant neurological, cardiac, and hepatic dysfunction. The phenotype of the following six variants: Gly26Arg, Trp50Arg, Leu60Arg, Del70-72, Leu75Pro and Leu64Pro is characterized by renal manifestations in association with extensive visceral amyloid deposits and hepatosplenomegaly. Clinical presentation is usually with hypertension and proteinuria between 18 and 55 years of age. Patients with the Leu75Pro variant have reportedly presented as late as their seventh decade and survived into their 90s.[50, 170] Progression of renal impairment is slow with a median time to ESRF

from presentation of eight years in one series. A large load in the liver frequently occurs in association with elevated serum ALP and gamma-glutamyl transpeptidase (GGT), but synthetic function is rarely affected and usually is a late feature after many years of progressive amyloid deposition, and fulminant hepatic failure is extremely rare.[171] Several ApoAI variants (Leu90Pro, Arg173Pro, Leu174Ser and Leu178His) are associated with skin and cardiac amyloid deposits with death usually occurring due to progressive cardiomyopathy within 10 years of diagnosis.[172-175]

It is thought that at least 50% of ApoAI is produced in the liver.[176] Liver transplantation has been associated with regression of extra-hepatic amyloid in a number of cases,[177] the reduction in supply of variant ApoAI by around 50% can be sufficient to alter the balance of amyloid deposition and its natural turnover in favour of regression. ApoAI amyloidosis is a slowly progressive disorder and reports following renal transplantation have shown remarkable graft survival, frequently exceeding 10-15 years despite histological evidence of recurrent amyloid in the transplanted organ.[176] Liver transplantation has been performed very rarely, but would appear to have the potential to benefit patients with extra-renal amyloidosis, notably peripheral nerve and cardiac involvement, or those with progressive liver dysfunction.

Apolipoprotein AII Amyloidosis (AApoAII)

Apo AII amyloidosis was first described by Weiss and Page in 1973.[178] It is the second most abundant HDL apolipoprotein. Four amyloidogenic mutations have been reported to date. Presentation is with proteinuria and progressive renal impairment. Age of onset tends to be earlier than Apo AI amyloidosis with cases

requiring renal replacement therapy by age 30-40 years. Russian and Spanish kindreds have reported later onset with presentation age 30 years and onset of renal replacement therapy at age 50 with the Stop78Arg mutation.[179,180] Outcome following transplantation has been reported to be excellent but the number of reported patients is very small. To date no patients with this form of amyloidosis have been reported in the UK.

Lysozyme Amyloidosis (ALys)

Lysozyme amyloidosis was first described by Pepys *et al* in 1993. Lysozyme is a bacteriolytic enzyme found in high concentrations in the liver, articular surfaces, saliva and tears. It is highly expressed in granulocytes, monocytes and bone marrow precursor cells. To date seven amyloidogenic mutations have been found: Ile56Thr, Phe57Ile, Trp64Arg, Asp67His, Trp112Arg, Tyr54Asn and D67G. Clinical presentation is with very slowly progressive renal failure, which usually presents in the third and fourth decades of life. Involvement of the liver, lymph nodes, GI tract, and spleen also occurs. Sicca syndrome due to salivary amyloid deposition is frequently reported in patients with Try64Arg and Asp67His variants, and lung and thyroid deposits have been reported in patients with Ile56Thr.[180] Renal decline can take decades, and the few reports of renal transplantation have been excellent, with reported graft function of over 15 years in some patients.

Therapeutic Targets

To date the objectives of therapy in amyloidosis have comprised supportive measures coupled with efforts to suppress production of the respective amyloidogenic precursor protein as previously discussed. The latter is frequently associated with gradual regression of existing amyloid deposits and preservation of vital organ function, and associated improvement in survival.[78, 181] Although great strides have been made in the management of AL and AA amyloidosis, this strategy still often fails and it has not been applicable in many other types of amyloid. This has led to the search for new drug therapies which apply a different approach to either inhibit amyloid deposition or enhance removal of established amyloid deposits.

Inhibiting Amyloid Fibrillogenesis

Highly sulphated glycosaminoglycans, particularly heparan and dermatan sulfate proteoglycans, are universal constituents of amyloid deposits and are thought to promote fibril assembly and aid in maintenance of the conformational changes associated with amyloidogenesis.[182, 183] Eprodisate (1, 3-propanedisulfonate) is a negatively charged, sulfonated molecule of low molecular weight that has structural similarities to heparan sulphate. It binds to the binding site on SAA to prevent its interaction with GAGs thus inhibiting the conformational change needed to render SAA amyloidogenic. In vivo studies have shown that Eprodisate inhibits the development of amyloid deposits in mouse models.[184,185]

Eprodisate remains the only drug to have been tested as a phase II/III multicentre, placebo controlled, double blinded study in amyloidosis.[186] Patients

with AA amyloidosis were stratified by the presence of nephrotic syndrome and treatment centre and then randomized either to Eprodisate or placebo twice daily for up to two years. Outcome measures were a composite end point of serum creatinine, creatinine clearance, and progression to ESRF or the patient's death. Secondary outcomes were the slope of creatinine clearance, change in proteinuria, improvement in diarrhoea and change in the amyloid content of abdominal fat. Unfortunately the study enrolled only 183 patients, this may have contributed to the failure to achieve the pre-specified primary endpoints although renal benefits were seen in the drug treated group. The hazard ratio for worsening disease with Eprodisate treatment was 0.58 (95% confidence interval, 0.37 to 0.93; P=0.02). The mean rate of decline in creatinine clearance was also significantly slower in the Eprodisate group than placebo; 10.9 compared to 15.6 ml per minute per 1.73 m^2 of body-surface area per year (P=0.02). Unfortunately the study failed to demonstrate a significant benefit from active therapy on progression to ESRF or risk of death, although there was a trend to benefit. A second clinical study is currently underway.

TTR amyloidosis has emerged as a particular focus for novel drug development, and several novel approaches are being pursued. The native soluble TTR molecule is a tetramer comprising two dimers that create and span a thyroid hormone-binding pocket. It is thought that a requisite step in the transformation of soluble TTR to its amyloid form is disruption of the normal TTR homotetramer into its monomeric components, which can auto-aggregate in the misfolded but highly ordered fibrillar conformation.[187] The approach pursued by Kelly and his colleagues has been to identify ligands that occupy the thyroid hormone-binding pocket and stabilize the structure in its native tetrameric form.[188] This has led to

the development of a novel drug compound tafamidis, which has shown promise in a randomized trial in hereditary TTR amyloidosis associated with the Met30 variant.[189] Our group at University College London (UCL) has identified other very potent stabilising compounds that simultaneously occupy both T4 binding sites in each tetrameric TTR molecule, confirmed by X-ray crystallographic analysis, which is irreversible under physiological conditions, and inhibited amyloidogenic aggregation more potently than other known ligands.[187] The hepatic origin of circulating TTR has encouraged development of RNA inhibiting approaches to treatment, given the preferential potential of targeting the liver with these new technologies. Both silencing RNA and anti-sense oligonucleotide approaches, which aim to reduce production of TTR, are being developed for clinical trials.

Targeting SAP

An alternative approach has been to target other components of amyloid deposits in order to destabilise fibrils. The normal plasma protein SAP is a universal constituent of amyloid deposits, its presence may mask the presence of amyloid deposits and inhibit effective clearance.[190] Further amyloid formation is inhibited in SAP knockout mice.[191] In 1984 Pepys identified its role as a therapeutic target,[192] which subsequently led to the development of R-1-[6-[R-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid (CPHPC), this drug acts by inhibiting the binding of SAP to amyloid. The activity of this palindromic agent relates to its ability to crosslink pairs of SAP molecules face to face which both results in rapid hepatic clearance, and completely occludes the binding face of the SAP molecule.[193] A preliminary clinical study of CPHPC in

several forms of amyloidosis confirmed that regular administration produced profound and sustained depletion of SAP. The drug was given to a number of patients for several years with no apparent adverse effects, though the magnitude of potential clinical benefit was not sufficiently large to be ascertained in this open, non-controlled study.[194] This drug is now available for use on compassionate grounds to a limited number of patients.

Immunotherapy

Antibodies that recognise a common fibril epitope have been raised and administered to mice,[195] with both experimentally created AL amyloidomas,[21] and systemic AA amyloidosis resulting in a reduced amyloid burden. Most recently the observation that CPHPC efficiently depletes SAP from the blood, but only very slowly from the amyloid deposits has enabled the development of an antibody directed at SAP. By using CPHPC to deplete the plasma of SAP followed by an anti-SAP antibody rapid clearance of experimentally induced amyloid deposits by macrophages can be seen, this approach is currently in translation for patients.[196]

Aims and Scope of the Thesis

The studies within this thesis are centred on the two organs which are most commonly involved; the heart and kidneys. Deposition of amyloid within these organs has a significant impact on patient morbidity and mortality. This thesis describes the disease phenotype within the heart and kidneys defined according to the underlying precursor protein. Different variants of amyloidosis have a predilection for different organ systems because of the underlying precursor protein which forms the fibril. Much of the work within this thesis describes the relationship between the fibril precursor protein and its effect on the disease phenotype.

Referral patterns to the NAC have changed in recent years and increasing numbers of patients with wild type transthyretin amyloidosis are being identified. The epidemiology of systemic amyloidosis has been little studied. In the first results chapter the burden of systemic amyloidosis in the English population is identified. Using death certificate data the proportion of deaths attributable to amyloidosis is described and the sensitivity of such data evaluated. By combining data from the office of national statistics (ONS) and data from the NAC database it is possible to estimate the proportion of patients seen at the NAC and extrapolate an estimated disease incidence.

The epidemiology of amyloidosis is changing, in recent years there has been a marked increase in patients who are diagnosed with ATTRwt and elderly patients with this disease are being increasingly referred for diagnosis and review at the NAC. The published literature provides limited information on small cohorts of patients with ATTRwt. There is little data on prognostic features and

natural history. Patients with ATTRwt frequently pose a diagnostic challenge. The diagnosis of amyloidosis rests on histological proof of amyloid and identification of the underlying fibril type, predominantly using immunohistochemical techniques. Because of the increasingly well characterised nature of cardiac amyloidosis on a variety of imaging modalities, there has been a shift away from confirming amyloid histologically prior to referral to the national centre. This poses the frequent question of how confident we can be in correctly identifying the type of amyloid using imaging and clinical features alone. The aims of this section of the thesis were therefore twofold; firstly to characterise the disease phenotype of ATTRwt; and secondly, to determine differences between ATTRwt and cardiac AL amyloidosis. Using a statistical model, the probability of the underlying diagnosis of ATTRwt is calculated in patients with isolated cardiac amyloid and an underlying clonal disorder, the most challenging group of patients to diagnose.

In chapter four the marked difference in disease phenotype between patients with cardiac AL amyloidosis and ATTRwt is highlighted. Abnormalities on the ECG appear common in both disease type, patients with ATTRwt appear to have a higher prevalence of atrial fibrillation. This theme is explored further in chapter five where Holter monitors are used with the aim to assess the frequency of dysrhythmias in patients with systemic amyloidosis and to identify whether arrhythmic activity is associated with disease severity or amyloid fibril type.

The final two results chapters concentrate on the disease phenotype of renal amyloidosis and the close relationship between the fibril precursor protein and patient outcome. Renal involvement is the most common presentation of AL amyloidosis and progression to ESRF is a frequent complication of the disease.

By utilising data from over 900 patients over a 21 year period, features at baseline which affect survival are identified and outcome following renal replacement therapy is assessed. Suppression of the precursor protein has been previously described in several studies to affect patient outcome but little data exists identifying the effect on organ function. The primary aim of chapter six is to identify factors associated with renal outcome. Suppression of the monoclonal light chain protein is shown to affect renal outcome and the dFLC (difference between he involved and uninvolved light chain) is validated in amyloidosis for the first time. Baseline factors which affect patient outcome and survival on dialysis are also described.

In the final chapter, renal transplant outcome in amyloidosis is assessed. There is currently no consensus on who is potentially eligible for listing on the deceased donor waiting list. Whilst patients who have received transplants with systemic amyloidosis are a highly selected group it is important to identify those who have achieved successful transplant outcomes and those who have developed recurrent disease and lost their grafts. Following on from the previous results in which a reduction in the precursor protein conferred a greater chance of a better renal outcome; the hypothesis was that suppression of the precursor protein would also confer a benefit to graft outcome, prior to and following renal transplantation. The importance of disease natural history is also highlighted with regard to graft outcome.

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Chapter Two: Materials and Methods

Declaration

I have designed the studies, carried out the data collection and the analysis of the data. I recruited the patients to the Holter monitor study, collected the data and performed the statistical analysis in my role as a clinical research fellow at the National Amyloidosis Centre, University College Medical School (Royal Free Campus). Several diagnostic methods were carried out by other individuals in the department they were as follows:

Histological and immunohistochemical analyses were performed by Janet Gilbertson and Toby Hunt.

Gene sequencing was performed by Dorota Rowczenio, Tonia Russell and Hadija Trojer.

Echocardiography was performed by Babita Pawarova, Carolyn McCarthy and Oliver Manalo.

^{123}I-SAP scintigraphy was performed by David Hutt and Dorothea Gopaul.

Holter monitoring was read by Elizabeth Collins and reports were reviewed by Dr Carol Whelan and Dr Dominic Rogers.

Measurement for biochemical and haematological data were performed by the Royal Free Hospital laboratory services.

Statistics advice was given by Loveleen Bansi for chapter 6 and Aviva Petrie from the Biostatistics Unit at the UCL Eastman Dental Institute for chapters 2 and 3.

Patients

All of the patients whose individual details are used within this thesis have been seen at the UK National Amyloidosis Centre. An access database has been maintained with details of all patients found to have amyloidosis. All patients included from the database provided explicit informed consent. Death certificate data entailing the number of deaths from amyloidosis in England was provided by the Office of National Statistics. Individual level data from this source was only provided in patients who had been seen at the NAC and therefore previously consented for their information to be used.

SAP Scintigraphy

SAP scintigraphy was performed in all patients at the initial assessment and at follow up assessment if clinically indicated for the monitoring of amyloid deposits. Female patients of child bearing age were asked to confirm that they were not pregnant. Each subject undergoing SAP scintigraphy received approximately 200µg of SAP with 190MBq of ^{123}I, the equivalent of 3.8 mSV of radiation. Thyroid uptake was blocked by the administration of 60mg of potassium iodide immediately prior to the study and 5 further doses were given over the following three days. Anterior and posterior imaging was performed at either 6 or 24 hours after injection using an IGE-Starcam gamma-camera (IGE Medical Systems, Slough, UK).

The classification of amyloid load was defined as ‘normal’ if there was no abnormal localisation of the tracer; ‘small’ when uptake in one or more organs was visible with normal intensity in the blood pool; ‘moderate’ when abnormal

uptake was seen within organs and the blood pool was diminished and 'large' when the blood pool signal was lost with adjustment of the grey scale to encompass the target organ.

Regression of amyloid was defined as reduction of the tracer within an affected organ and/or an increase in the background blood pool. Amyloid progression was defined as an 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.

Cardiac Assessment

SAP scintigraphy is not used as part of the cardiac assessment. The large blood-pool within the chambers of the heart and cardiac motility mean that amyloid is poorly visualised using this technique. A combination of electrocardiogram, echocardiogram and cardiac biomarkers are used routinely at the NAC to evaluate whether there is cardiac amyloid. CMR is not available at the centre. Where patients have previously had a CMR the images were viewed on CDs by Dr Jason Dungu who evaluated whether the changes were consistent with amyloid. In patients where the diagnosis of cardiac amyloid was in doubt CMR was arranged either through the referring physician or patients were scanned at the heart hospital.

Functional Evaluation

Functional evaluation of patients performance status was evaluated in the clinic by the Eastern Co-operative Group (ECOG) performance status[197] (Table 2.1)

This has been used historically as a guide for cancer patients to determine whether patients will tolerate treatment, whether dose adjustment may be necessary or whether palliation may be a suitable option. The New York Heart Association Classification (NYHA) was also used in patients attending the clinic, this classification provides a useful standardised way of classifying the extent of heart failure symptoms in patients (Table 2.2).[198]

Table 2.1 Definition of Eastern Co-operative Group Performance Status

Grade	**Summary**	**Description**
0	Normal	No restriction to carrying out normal activities
1	With effort	Ambulatory, able to do light work. Restricted only in strenuous activity
2	Restricted	Self caring and ambulatory but unable to carry out work
3	Dependent	Capable of limited self-care, confined to bed or chair for over 50% of waking hours
4	Immobile	Unable to carry out self-care, completely confined to bed or chair

Table 2.2 Definition of New York Heart Association Classification

NYHA Class	**Summary**	**Description**
I	Normal	No limitation of physical activity. Ordinary physical activity does not cause shortness of breath or undue fatigue
II	Mild	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation or dyspnoea
III	Moderate	Marked limitation of physical activity. Comfortable at rest but less than ordinary activity causes fatigue, palpitation or dyspnoea
IV	Severe	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased

Echocardiography

Echocardiography was performed in all patients with two-dimensional and M-mode settings using a GE Vivid 7 system. Parasternal long axis and apical long axis views were most commonly used. Evaluation of left ventricular wall thickness, left ventricular diastolic function, left ventricular systolic function and atrial diameter were measured using defined criteria from the British Society of Echocardiography (http://www.bsecho.org). Left atrial area was measured using criteria defined by the American Society of Echocardiography (http://www.asecho.org).

Electrocardiogram

All patients had a standard 12 lead ECG at each visit. Standard sensitivity of 1cm per mV was used. Historical data has shown an association between a low voltage ECG and increased cardiac mass on echocardiography with cardiac amyloidosis.[199] The current amyloidosis consensus criteria define low voltage ECG as QRS complexes of <5mm in all limb leads.[60] These criteria have been used to define low QRS complexes on the ECG. Total QRS score and mean QRS in leads II/III/AVF and V4/V5/V6 have also been reported.

Holter Monitoring

Twenty-four hour electrocardiographic monitoring (Holter monitoring) was performed using a portable cassette recorder with three lead placements (Spacelabs Healthcare Lifecard CF). Analysis of the 24 hour records was performed by an experienced electro physiologist using Pathfinder Digital

software V8.701 (Spacelabs). Two consultant cardiologists reviewed the reports. Referring physicians were contacted with the results of significant results and changes to management were recommended based on current clinical practice for cardiac rhythm disturbances.

Criteria for Diagnosis of Amyloid and Definition of Organ Response

The definition of organ involvement and organ response was defined according to consensus criteria in combination with SAP scintigraphy (Table 2.3).[60]

Table 2.3 Definition of Organ Involvement and Organ Response

Organ	Definition of Organ Involvement	Definition of Organ Response
Heart	Echocardiogram: Mean wall thickness >12mm and no other cardiac cause or CMR showing late gadolinium enhancement	Mean IVSd decreased by 2mm, 20% improvement in EF, improvement by 2 NYHA classes without an increase in diuretic use and no increase in wall thickness
Kidneys	24 hour non Bence Jones Proteinuria >0.5g, or uptake on SAP scintigraphy	50% reduction in proteinuria (at least 0.5g/day) creatinine and creatinine clearance must not worsen by 25% over baseline
Liver	SAP scintigraphy	50% decrease in abnormal ALP or reduced organ uptake on SAP scintigraphy
Spleen	SAP scintigraphy	Reduced organ uptake on SAP scintigraphy
Adrenal	SAP scintigraphy	Reduced organ uptake on SAP scintigraphy
Soft Tissue	Tongue hypertrophy, periorbital bruising, spontaneous bruising, pseudo hypertrophy, lymphadenopathy, carpal tunnel syndrome	Clinical assessment of improvement
Gastrointestinal Tract	Direct biopsy verification with symptoms	
Lung	Direct biopsy verification with symptoms, interstitial radiographic pattern	Radiographic evidence of improvement in pulmonary interstitial amyloid (rare)
Peripheral Neuropathy	Symmetrical sensorimotor peripheral neuropathy in the lower limbs	Clinical assessment
Autonomic Neuropathy	Impotence, diarrhoea or constipation, early satiety and/or impaired bladder emptying without other overt cause. Orthostatic hypotension (>20mmHb fall in systolic BP)	Clinical Assessment

Histology

Congo Red Staining

Formalin fixed de-paraffinised tissue sections 6-8µg thick were rehydrated, and counterstained with haematoxylin under running tap water. Sections were then stained using the alkaline-alcoholic Congo-red method as previously described by Puchtler *et al.*[200] A series of ascending ethanol concentrations to xylene were used to dehydrate the sections which were then mounted in DPX mounting medium. Stained slides were then viewed in bright field and under cross polarised light. Positive controls were obtained from a known Congo-red positive block validated by laser micro dissection and mass-spectrometry based proteomic analysis which was always processed in parallel.

Immunohistochemistry

The amyloid type was then characterised by immunohistochemical staining. Formalin fixed de-paraffinised 2µm sections of amyloidotic tissue were used. Sections were washed with water and endogenous peroxidise activity was quenched by incubation in aqueous (0.3%) hydrogen peroxide (H_2O_2) for 30 minutes. They were then rinsed again in phosphate-buffered saline (PBS) containing 0.05% Tween (Calbiochem). Prior to the application of antisera, non specific tissue binding was abolished by incubation for a further 30 minutes in normal non-immune serum from the species providing the secondary antibody (Vector Part of the ImmPRESS Kit). Sections were then incubated overnight with primary antisera at 4^{o}C. They were rinsed with PBS containing 0.05% Tween (Calbiochem) and labelled with secondary antibodies. Sections were washed in

PBS and bound enzyme-antibody bound complexes were then visualised using a metal-enhanced DAB (Fisher Scientific solution).

A panel of anti-human monospecific antibodies reactive with: SAA (Eurodiagnostica, Huntington UK) AL kappa, lambda, transthyretin and lysozyme (Dako Ltd, Denmark House Ely UK), Apolipoprotein AI (Genzyme Diagnostics) and fibrinogen Aα chain (Calbiochem) were used where appropriate. Congo red overlay was used in duplicate sections. Immunohistochemically stained sections were counterstained in haematoxylin, 'blued' under running tap water and stained with Congo-red.[201]

For TTR staining, pre-treatment was performed for enhanced antigen retrieval using 10 minute incubation with 1% sodium periodate, slides were then washed and further incubated for 10 minutes with 0.1% sodium metabisulphate, washed again and incubated for 5 hours at room temperature with 6M Guanadine in 0.9% sodium chloride.

Immunoassays

Serum Amyloid A Protein

SAA levels were measured using latex nephelometry (BNII autoanalyser Dade, Behring Marbury, Germany).[81] The lower limit of detection is 0.7mg/L. Standardisation was based on WHO international reference standards 1987.[202]

Serum Free Immunoglobulin Light Chain Assay

Both kappa and lambda serum free immunoglobulin light chains (FLC) were measured using a latex-enhanced immunoassay (The Binding Site, Birmingham, UK) on a ehring BNII autoanalyser (Dade Behring, Marburg, Germany).[203-205] Antibodies are directed against FLC epitopes hidden within whole immunoglobulin molecules. The sensitivity of the assay is <5mg/L. Sera from 100 healthy blood donors were tested in order to determine the reference range. 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 ((%% CI, 8.91-29.87mg/l) respectively. The mean kappa/lambda ratio was 0.7 (95% CI, 0.37-0.95). An abnormally high kappa or lambda light chain value or abnormal ratio in the context of preserved renal function was used as part of the assessment of an underlying clonal disorder in chapter four. In patients with renal impairment the ratio alone was used. The definitions of haematologic response are outlined in Table 2.4.

Light chains are metabolised in the kidneys. Polyclonal free light chain levels rise in renal failure[206] which makes interpretation of absolute levels of serum free light chains difficult to interpret. If the ‘normal’ ratio of kappa/lambda

is estimated to be 1:1, the amount of monoclonal light chain can be estimated by subtracting the uninvolved light chain from the involved light chain, a method previously validated in myeloma.[207] This method has been used when calculating the light chain response in chapters six and seven.

Table 2.4 Haematologic Response Criteria[60]

Clonal Response	Criteria
Complete Response	Serum and urine negative for a monoclonal protein by immunofixation, normal free light chain ratio
Partial Response	If serum paraprotein >0.5g/dL, a 50% reduction If light chain in the urine with a visible peak and >100mg/day and 50% reduction If free light chain >10mg/dL (100mg/L) and 50% reduction
Progression	From CR, any detectable monoclonal protein or abnormal free light chain ratio (doubling of light chain) From PR or stable response, 50% increase in serum paraprotein to >0.5g/dL or 50% increase in urine paraprotein to >200mg/day Free light chain increase of 50% to >10mg/dL (100mg/L)
Stable	No CR, no PR, no progression

Gene Sequencing

Genotyping was performed in patients with suspected hereditary amyloidosis where appropriate. Whole blood taken in an EDTA tube was frozen and stored for gene sequencing as required. Genomic DNA was isolated by a rapid method.[208] The blood was added to NH_4CL and spun, the sample was then re-suspended in 0.9% NaCl and re-spun. It was then suspended again in 0.05M NaOH, incubated, cooled and neutralised with 1M Tris pH8. Polymerase chain reaction (PCR) using 'Ready-To-Go' tubes (GE Healthcare) were used to amplify the coding regions for the following genes: transthyretin (exons 2,3 and 4), apolipoprotein AI (exons 3 and 4) and fibrinogen A α-chain (exon 5). HotStar Taq DNA Polymerase kit

(Qiagen) was used for the lysozyme gene (exon 2). The primers used as part of the PCR process are outlined in Table 2.5.

Table 2.5 Primers Used in the PCR Process for Genotyping Hereditary Amyloidosis

Gene (exon)	Forward primer sequence	Reverse primer sequence
Transthyretin (2)	5'-TTTCGCTCCAGATTTCTAATAC-3'	5'-CAGATGATGTGAGCCTCTCTC-3'
Transthyretin (3)	5'-GGTGGGGGTGTATTACTTTGC-3'	5'-TAGGACATTTCTGTGGTACAC-3'
Transthyretin (4)	5'-GGTGGTCAGTCATGTGTGTC-3'	5'-TGGAAGGGACAATAAGGGAAT-3'
Apolipoprotein (3)	5'-GGCAGAGGCAGCAGGTTTCTCAC-3'	5'-CCAGACTGGCCGAGTCCTCACCTA-3'
Apolipoprotein (4)	5'-CACTGCACCTCCGCGGACA-3'	5'- CTTCCCGGTGCTCAGAATAAACGTT-3'
Fibrinogen (5)	5'-AGCTCTGTATCTGGTAGTACT-3'	5'- ATCGGCTTCACTTCCGGC-3'
Lysozyme (2)	5'-GTTATATTGTTCGTTGGTGT-3'	5'- CATTTGTATTGAGTCTCAATTC-3'

Statistical Analysis

Statistical analyses were performed using Graph Pad Prism (Version 4 and 5), SPSS 15 (SPSS Inc, Chicago III) and Stata version 11 (Statacorp).

Individual statistical methods are discussed separately in each results chapter.

Results Section One: Epidemiology

Chapter Three: Systemic Amyloidosis in England: An Epidemiological Study

Introduction

Amyloidosis has been loosely estimated to cause about 0.5-1.0 deaths per thousand in the UK.[209] There are almost no published epidemiological studies of amyloidosis in the medical literature; the most comprehensive being a study conducted by Kyle and colleagues at the Mayo Clinic of the general population residing in the surrounding area of Olmstead County, USA.[25] They reported an incidence of AL amyloidosis of 5.1-12.8 per million person-years, using data from a centralized system recording virtually all medical, surgical and pathological diagnoses of the county residents. Twenty one individuals from Olmstead County were diagnosed with AL amyloidosis between 1952 and1992. The overall age and sex adjusted annual incidence rate was reported to be 8.9 per million person-years. The authors extrapolated that approximately 2225 new cases may occur annually across the USA, but this has not been verified.

The NAC database effectively serves as a national registry of the disease. This database has enabled the natural history and response to therapeutic interventions of the various amyloidosis syndromes to be studied in large cohorts of patients.

Studies using data from death certificates have been conducted in several rare diseases to estimate the burden of mortality[210-212] and indirectly estimate incidence of disease. Systemic amyloidosis lends itself to this approach since it is

an incurable and usually rapidly fatal disorder;[45,86,177,213] it has specific pathological features enabling definitive diagnosis through biopsy, and which are overt in hitherto undiagnosed cases at autopsy. Further, given the requirement for histological diagnosis, it is probable that false positive recording of amyloidosis on death certificates is exceptionally rare.

The aim of this study was to estimate the incidence of amyloidosis in England based on analysis of two data sets: reported death certification from the Office of National Statistics (ONS) and information on referrals and deaths held on the NAC database.

Methods

Office of National Statistics Death Certificate Data

The number of deaths registered in each calendar year for people living in England and the proportion of those deaths in which the word 'amyloidosis' appeared anywhere on the death certificate, as defined by the International Classification of Diseases ninth revision (ICD-9) code for the year 2000 and tenth revision (ICD-10) code from 2001 onwards (Table 3.1), were obtained from the ONS. All data was fully anonymised.

Table 3.1 Amyloidosis deaths – International Classification of Diseases Ninth (ICD-9) and Tenth Revision (ICD-10)

Cause of Death	**ICD-9 Code**
Amyloidosis	277.3
Cause of Death	**ICD-10 Code**
Non-neuropathic heredofamilial amyloidosis	E85.0
Neuropathic heredofamilial amyloidosis	E85.1
Heredofamilial amyloidosis, unspecified	E85.2
Secondary systemic amyloidosis	E85.3
Organ-limited amyloidosis	E85.4
Other amyloidosis	E85.8
Amyloidosis, unspecified	E85.9

Data from the National Amyloidosis Centre Database

All English residents diagnosed to have systemic amyloidosis between 2000 and 2008 whose details were held on the NAC databases were identified and their details were provided to ONS. Death data was returned from ONS for all matched individuals, including date and cause of death as listed in parts IA, IB, IC, and II of the respective death certificates. Matching of individuals between

the ONS and NAC databases required agreement of name, date of birth and unique NHS number. The type of amyloid and date of diagnosis was obtained for each case from the NAC database and survival from the first assessment at NAC was calculated. Survival data were censored on January 1st 2012.

Office of National Statistics Estimate of Population

Estimates of the population in England were taken from the ONS website 'Mid-1971 to Mid-2010 Population Estimates: Quinary age groups for Constituent Countries in the United Kingdom; estimated resident population' released on 21st December 2011. Estimates are of the usually resident population on 30th June of the reference year and reflect administrative boundaries that were in place on that day.

Statistics

The study was approved by the Royal Free Hospital ethics committee. Graph Pad Prism version 5 was used for statistical analyses. Patient survival was estimated by Kaplan-Meier Analysis using SPSS version 20. The log-rank test was used to compare differences between stratified Kaplan-Meier survival curves. Statistical significance was achieved if $P < 0.05$.

Results

Accuracy of Death Certificate Data

Summary statistics on the total number of deaths attributable to systemic amyloidosis between 2000 and 2008 are shown in Table 3.2. Amyloidosis appeared on the death certificates of 2543 English individuals during this period. There were 1143 deaths over the same period among patients with systemic amyloidosis who were registered on the NAC database, 903 (79%) of whom had amyloidosis reported on their death certificate. Amyloidosis was recorded in Part 1A, 1B, and 1C of the death certificate in 261 (28.9%), 372 (41.2%) and 96 (9.6%) respectively, and in Part 2, which identifies conditions contributing to the cause of death, in 174 (19.2%) individuals (Figure 3.1). The proportion of patients registered on the NAC database in which amyloidosis was stated on the death certificate did not vary significantly over the study period. However, there was an association between amyloid type and the frequency with which amyloidosis appeared on the death certificate (Table 3.3, P=<0.001); 83% percent of patients with AL amyloidosis had amyloidosis on their death certificate, compared to 74% of those with hereditary amyloidosis and ~62% of those with AA and transthyretin amyloidosis.

Figure 3.1 Position of amyloidosis on death certificates among patients who attend the National Amyloidosis Centre.

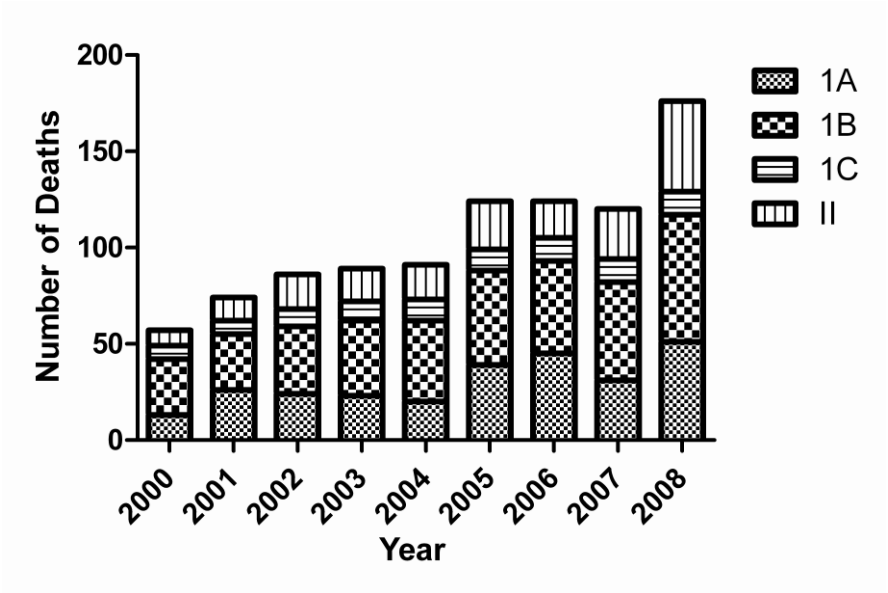


Table 3.2 Total number of deaths in England with amyloidosis recorded anywhere on the death certificate and total number of patients from England reviewed at the National Amyloidosis Centre between 2000 and 2008

Year	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**Total**
Data from Death Certificates (ONS)										
Amyloidosis reported as the underlying cause of death (% of total certificates with amyloidosis stated)	128 (58)	154 (63.4)	171 (60.8)	153 (58.6)	165 (62.9)	197 (62.5)	174 (61.7)	208 (63.8)	217 (61.5)	1567 (61.6)
Amyloidosis anywhere on the death certificate	220	243	281	261	262	315	282	326	353	2543
Total deaths in England	503024	497878	500795	504127	480716	479678	470326	470721	475763	4383028
Proportion of deaths with amyloid mentioned on death certificate (per thousand deaths)	0.44	0.49	0.56	0.5	0.54	0.66	0.60	0.69	0.74	0.58
Data from NAC Database										
Number of deaths in patients seen at NAC	66	84	98	110	116	144	151	157	217	1143
NAC deaths with amyloid on certificate N (%)	55 (83.3)	69 (82.1)	82 (83.7)	87 (79.1)	87 (75)	119 (82.6)	118 (78.1)	116 (73.9)	170 (78.3)	903 (79.0)
Total deaths with amyloid on death certificate and confirmed NAC deaths with no mention of amyloid	231	258	297	284	291	340	315	367	400	2783
National proportion of deaths with amyloid from death certificate and NAC confirmed cases (per thousand deaths)	0.46	0.52	0.59	0.56	0.61	0.71	0.67	0.78	0.84	0.63
Number of patients with amyloid on death certificate not seen at NAC N (%)	165 (75)	174 (71.6)	199 (70.8)	174 (66.6)	175 (66.8)	196 (62.2)	164 (58.2)	210 (64.4)	183 (51.8)	1640 (64.5)

Table 3.3 Number of patients seen at the National Amyloidosis Centre with amyloid on their death certificate, stratified by amyloid fibril type

Amyloid mentioned on death certificate	AL Amyloidosis		AA Amyloidosis		Hereditary Amyloidosis		Senile Systemic Amyloidosis	
	No	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**
2000	4 (8)	46 (92)	6 (60)	4 (40)	1 (16.7)	5 (83.3)	0	0
2001	7 (10.3)	61 (89.7)	5 (38.5)	8 (61.5)	3 (100)	0	0	0
2002	12 (15.3)	72 (84.7)	3 (42.9)	4 (57.1)	1(14.3)	6 (85.7)	0	0
2003	16 (18.4)	71 (81.6)	5 (29.4)	12 (70.6)	0	4 (100)	2 (100)	0
2004	16 (18.2)	72 (81.8)	6 (54.6)	5 (45.4)	2 (22.3)	7 (77.7)	5 (62.5)	3 (38.5)
2005	22 (18.1)	100 (81.9)	2 (14.3)	12 (85.7)	1 (12.5)	7 (87.5)	0	0
2006	23 (19.3)	96 (80.7)	6 (31.6)	13 (68.4)	4 (40)	6 (60)	0	3 (100)
2007	27 (22.3)	94 (77.7)	11 (47.8)	12 (52.2)	1 (16.7)	5 (83.3)	2 (28.6)	5 (71.4)
2008	33 (9)	139 (81)	6 (37.5)	10 (62.5)	6 (30)	14 (70)	2 (22.3)	7 (77.7)
Total (%)	160(17.5)	751(82.4)	50 (38.5)	80 (61.5)	19 (26)	54 (74)	11 (37.9)	18 (62.1)

Cause of Death Among Patients with Systemic Amyloidosis

The primary cause of death was likely directly related to amyloidosis among 127/174 (73%) patients, and was listed as follows: 12 end stage renal failure (ESRF), 22 heart failure, 56 sepsis, 14 multi-organ failure, 17 myeloma/lymphoma, 5 pulmonary embolism and 1 nephrotic syndrome. A further 32 (18%) deaths among these 174 individuals were possibly related to the amyloidosis and the primary causes were listed as follows: 17 cerebrovascular accidents (CVA), 9 myocardial infarctions (MI), 4 sudden cardiac deaths, 1 ruptured abdominal aortic aneurysm and 1 bowel perforation. Only 15/174 (9%) deaths appeared unrelated to systemic amyloidosis, 14 from unrelated malignancies and 1 from chronic obstructive pulmonary disease (COPD).

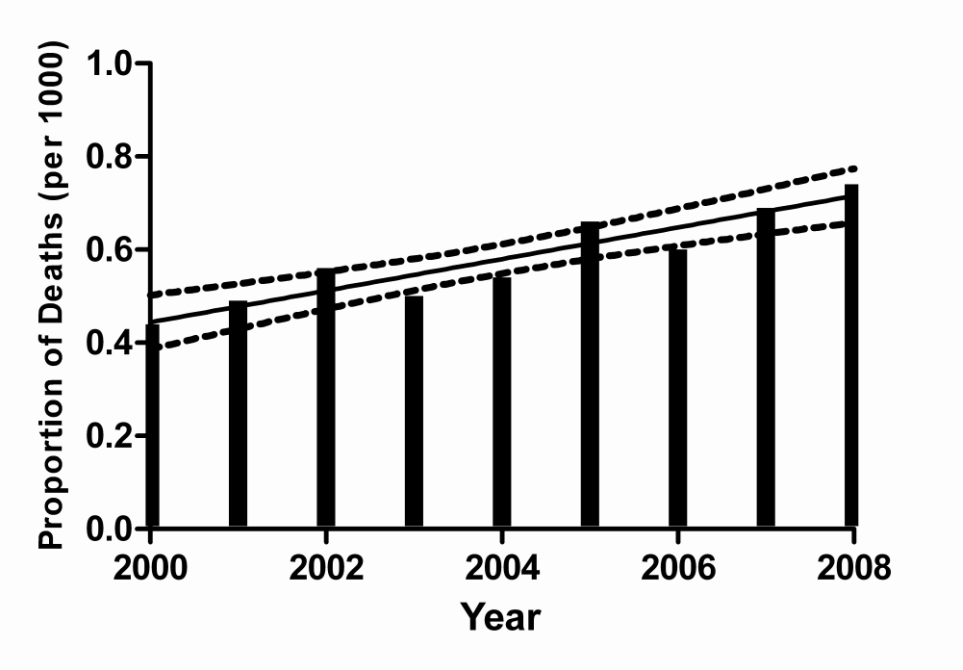
Of the 240 individuals with systemic amyloidosis who were followed at the NAC but did not have amyloidosis stated on their death certificate, 179 (75%) had primary causes of death which were likely related to their amyloid. Among the remaining 61/240 (25%) patients the primary cause of death was listed as follows: 26 ischaemic heart disease or heart failure, 13 'other' malignancy, 10 CVA, 3 COPD, 2 sepsis, and 1 each from drug overdose, end-stage renal failure, bowel obstruction, bowel ischaemia, pelvic abscess, peritonitis, and vasculitis. Taken together, these results suggest that amyloidosis contributed directly to death in more than 90% of patients with systemic amyloidosis.

Estimate of Deaths Attributable to Amyloidosis in England

According to death certificate data, 0.58 per thousand deaths were attributable to amyloidosis in England between 2000 and 2008. Over the 9 year period, there

was a significant increase in the proportion of death certificates on which amyloidosis was included as a cause of death (Figure 3.2, R^2 0.86, P=<0.001). Since 21% of patients who were known to the NAC with systemic amyloidosis did not have 'amyloidosis' mentioned on their death certificates, we estimated that at least the same proportion of cases may not be reported on death certificates in England generally. Assuming 21% of deaths from systemic amyloidosis were not reported on English death certificates between 2000 and 2008, the total number of individuals who died from systemic amyloidosis in England during this time period was 3077 (2543 (79% reported) + 534 (21% unreported)), representing 0.7 per 1000 deaths in the country. Taking only the data for 2008 and using the same calculation (i.e., assuming 21% deaths from systemic amyloidosis were not reported as such), the total number of deaths from systemic amyloidosis in England in 2008 was 429, representing 0.9 per 1000 (429/475763) deaths.

Figure 3.2 Proportion of death certificates from England on which amyloidosis was included as a cause of death.



Estimate of Incidence and Prevalence of Amyloidosis in England

Referrals to NAC of patients with systemic amyloidosis (Table 3.4) doubled over the nine year study period (R^2 0.8167, P=0.0008), from 0.29 per 100000 population in 2001 to 0.51 per 100000 population in 2008. The incidence increased with age and peaked between 60 and 79 years. A proportion of patients with systemic amyloidosis are not referred to the NAC, and although this proportion appeared to fall substantially between 2000 and 2008 (Table 3.2), it is only possible to reliably estimate the minimum disease incidence from NAC data. The two-fold increase in referrals to the NAC between 2000 and 2008 is likely to reflect a combination of increased awareness of the disease and demand for NAC services, as well as improvement in diagnosis. In 2008, 48.2% of the individuals in England in whom amyloidosis was recorded on their death certificate had been assessed at the NAC. Extrapolation to the population of England generally

suggests an annual incidence in 2008 of 0.1/100000 persons. Table 3.5 shows the number of patients in England diagnosed with different types of systemic amyloidosis at the NAC. Systemic AL amyloidosis is the most prevalent, with a minimum overall incidence of 0.3 cases per 100000 population in 2008, which increases to 0.5 per 100000 when the estimated 51.8% of patients who were not seen at the NAC are included.

According to NAC data, there were 435 individuals living in England with systemic amyloidosis in 2000 and 1051 individuals living with the disease in 2008. This apparent increase in prevalence is likely, in part, to reflect improved survival (median (CI) 27.6 (16.4 - 38.9) months in 2000 vs. median (CI) 45 (43.2 – 46.7) months in 2008; log rank test for trend P=0.02) (Table 3.6) as well as changing referral patterns.

Table 3.4 Estimated incidence of systemic amyloidosis in England by age based purely on confirmed diagnoses among patients attending the National Amyloidosis Centre

Year		0-19 years	20-29 years	30-39 years	40-49 years	50-59 years	60-69 years	70-79 years	80+ years	Total newly diagnosed cases (Overall estimated Incidence)	Total alive at NAC/ Prevalence** (%)	Total Pop
2000	New cases at NAC (incidence*)	0 (0)	1 (0.01)	2(0.02)	22 (0.3)	31 (0.5)	48 (1.0)	20 (0.5)	5 (0.2)	129 (0.26)	435/0.88	
	Population	12,357900	6,350000	7,736000	6,511400	6,083300	4,570300	3,626900	1,997300			49,233100
2001	NAC	0 (0)	2 (0.03)	15 (0.2)	13 (0.2)	33 (0.5)	44 (0.9)	36 (1.0)	1 (0.05)	144 (0.29)	498/1.00	
	Population	12,327700	6,307100	7,769600	6,616900	6,197600	4,555600	3,597600	2,077900			49,450000
2002	NAC	0 (0)	0 (0)	9 (0.1)	10 (0.1)	44 (0.7)	62 (1.0)	44 (1.0)	6 (0.28)	175 (0.35)	581/1.17	
	Population	12,334200	6,245000	7,759700	6,743000	6,280600	4,574000	3,577600	2,135000			49,649100
2003	NAC	0 (0)	1 (0.01)	6 (0.07)	8 (0.1)	46 (0.7)	62 (1.0)	51 (1.0)	9 (0.41)	183 (0.36)	646/1.29	
	Population	12,358000	6,232900	7,694600	6,890900	6,301500	4,657800	3,565300	2,172300			49,873300
2004	NAC	0 (0)	6 (0.09)	4 (0.05)	20 (0.2)	41 (0.6)	61 (1.0)	41 (1.0)	12 (0.54)	185 (0.37)	710/1.41	
	Population	12,365100	6,318600	7,575000	7,040800	6,309100	4,742600	3,555600	2,203000			50,109800
2005	NAC	1 (0.008)	1 (0.01)	6 (0.08)	16 (0.2)	40 (0.6)	75 (1.0)	47 (1.0)	9 (0.40)	195 (0.38)	788/1.56	
	Population	12,350400	6,488200	7,459400	7,217900	6,313100	4,836800	3,563900	2,236300			50,466000
2006	NAC	0 (0)	3 (0.04)	11 (0.1)	15 (0.2)	34 (0.5)	62 (1.0)	62 (1.0)	13 (0.57)	200 (0.39)	853/1.68	
	Population	12,337600	6,641400	7,313000	7,369200	6,319700	4,927200	3,579100	2,277000			50,764200
2007	NAC	0 (0)	0 (0)	4 (0.05)	18 (0.2)	44 (0.7)	86 (1.0)	89 (2.0)	21 (0.90)	262 (0.51)	966/1.89	
	Population	12,351800	6,826500	7,142600	7,501100	6,217700	5,138800	3,607600	2,320100			51,106200
2008	NAC	3 (0.02)	3 (0.04)	10 (0.1)	17 (0.2)	50 (0.8)	83 (1.0)	82 (2.0)	19 (0.8)	267 (0.51)	1051/2.04	
	Population	12,360600	6,988300	7,014300	7,588900	6,183100	5,323800	3,650400	2,355200			51,464600

*Incidence per hundred thousand individuals of newly diagnosed cases per year, **prevalence; total number of people alive with the disease who have been seen at the NAC per hundred thousand population

Table 3.5 Estimated age-adjusted annual incidence in 2008 of each amyloid type per hundred thousand population in England assuming that all patients with amyloidosis are seen at the NAC

Number of patients (annual incidence per hundred thousand patients)				
Age Range	**AL Amyloidosis**	**AA Amyloidosis**	**Senile Systemic Amyloidosis**	**Hereditary Amyloidosis and Other**
0-19	0	3 (0.03)	0	0
20-29	0	3 (0.04)	0	0
30-39	1 (0.01)	6 (0.08)	0	3 (0.04)
40-49	11 (0.1)	5 (0.06)	0	1 (0.01)
50-59	38 (0.6)	7 (0.1)	0	5 (0.08)
60-69	59 (1.0)	14 (0.2)	1 (0.02)	8 (0.1)
70-79	54 (1.0)	9 (0.2)	10 (0.3)	9 (0.2)
80+	11 (0.5)	1 (0.05)	7 (0.3)	0
Total	174 (0.3)	48 (0.08)	18 (0.03)	26 (0.04)

Table 3.6 Kaplan Meier survival from the date of diagnosis among patients diagnosed with amyloidosis at the NAC by individual year of diagnosis

Median Survival (Months)		
Year of diagnosis	**Estimate**	**95% CI**
2000	27.7	16.4-38.9
2001	33.2	16.7-49.7
2002	17.4	11.3-23.5
2003	32.3	20.5-44.1
2004	27.5	16.2-38.8
2005	29.2	18.5-39.8
2006	36.0	20.5-51.6
2007	33.9	23.1-44.7
2008	45.0	43.2-46.7
Overall	31.8	27.4-36.2

Log Rank (Mantel Cox) for trend P = 0.023

Regional Differences in Death Rates and Referrals to the NAC

The regional differences across England in reported deaths from amyloidosis in 2008 are shown in Table 3.7. The proportion of deaths ranged from a minimum of 0.55/1000 in Yorkshire and the Humber to a maximum of 0.97/1000 in the South West. There were significantly less deaths reported in Yorkshire and the Humber compared to the East of England, South Central and the South West ($P<0.01$). There was no correlation between the proportion of deaths from amyloidosis and distance from the NAC. The number of new referrals to the NAC did vary between strategic health authorities (SHAs) however, with a greater number of patients being referred from SHAs located closer to the NAC (Figure 3.3). It would appear that nearly all patients who died from systemic amyloidosis in the London and the East of England regions had been seen at the NAC. These regions are therefore likely to offer the greatest accuracy for estimating disease incidence. The minimum estimated disease incidence in these two regions respectively, based on new patient referrals to the NAC, is 0.73 and 0.56/100000 population, corroborating earlier calculations of disease incidence.

Figure 3.3 Apparent incidence of amyloidosis in 2008 stratified by Strategic Health Authority, derived solely from new referrals to the National Amyloidosis Centre (NAC). The incidence appears to fall as distance from the NAC increases. ($R^2 = 0.64$, P = 0.005).

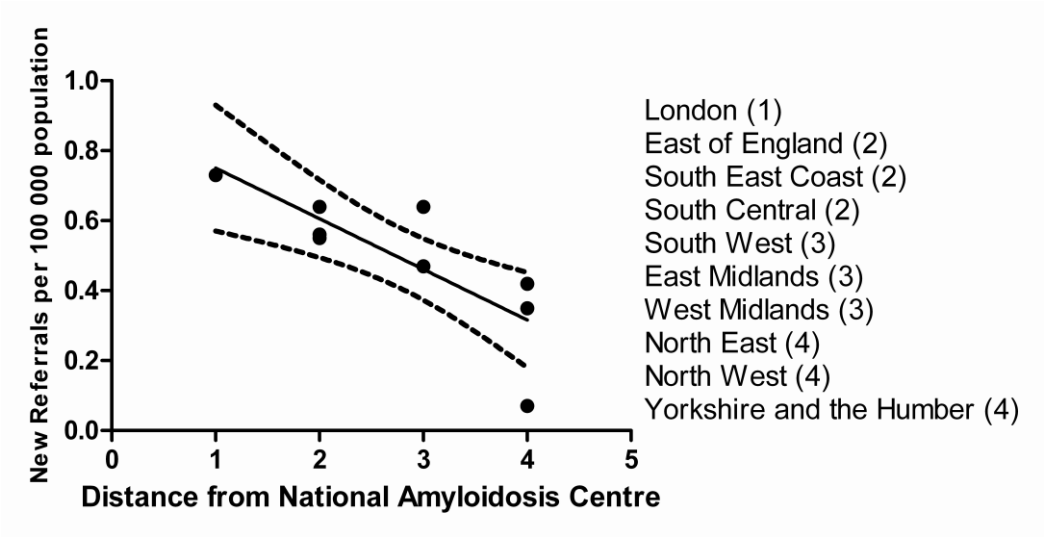


Table 3.7 Total Deaths and NAC Deaths from Amyloidosis and Incidence Based on New Referrals to the NAC in 2008 by Strategic Health Authority

Strategic Health Authority	ONS total deaths	ONS population estimates	Amyloidosis on death certificate	Proportion of Deaths due to Amyloidosis (per thousand)	Deaths from Amyloidosis (per 100000 population)	New patients diagnosed with amyloid at NAC	Incidence* of Amyloidosis per 100 000	Number of deaths in NAC patients
North east	27386	2,570600	20	0.73	0.77	2	0.07	4
North west	70740	6,874100	45	0.63	0.65	24	0.35	24
Yorkshire and the Humber	50539	5,217500	28	0.55	0.54	22	0.42	13
East midlands	42296	4,429400	25	0.59	0.56	21	0.47	8
West midlands	52318	5,408400	33	0.63	0.61	35	0.64	13
East of England	52689	5,717400	49	0.93	0.85	32	0.56	50
London	50476	7,668300	42	0.83	0.54	56	0.73	38
South east coast	42537	4,309400	26	0.61	0.6	24	0.55	16
South central	33380	4,059100	33	0.98	0.81	26	0.64	24
South west	53402	5,210400	52	0.97	0.99	25	0.47	27
Total	475763	51,464600	353	0.74	0.68	267	0.52	217

*Incidence of Amyloidosis – based on newly diagnosed NAC cases

Discussion

Information regarding the epidemiology of systemic amyloidosis is scarce. The most robust study by Kyle *et al* reported the incidence to be 8.9 per million person years. This study used patient record data from Olmstead County, USA to identify patients diagnosed with the disease between 1950 and 1989, and incidence was extrapolated on the basis of population data for the whole of the USA. The authors noted an increase in incidence across the time period.[25] The findings from the Olmstead County study have not been validated or updated; furthermore, the epidemiology of systemic amyloidosis has not previously been studied in England. A report from Boston University used death certificate data to estimate the number of deaths from systemic AL amyloidosis. Although the diagnostic criteria for AL amyloidosis were somewhat unreliable in this study, the estimated incidence based on mortality data was 4.5/100000.[26] Imaizumi *et al* used death certificates to estimate the rate of death from systemic amyloidosis in Japan, expressed as the number of cases with amyloid on death certificates per 100000 population alive during that year. They reported an increase in death rate from amyloid among males from 0.022/100000 in 1969 to 0.178/100000 in 1992.[214]

Death certificates have previously been used to estimate incidence in a number of rare diseases.[210] Death certificate data is deemed a reliable way of estimating disease incidence only in conditions which are well defined, rarely misdiagnosed, and consistently fatal without changing survival.[210] The sensitivity of mortality data for predicting disease incidence in systemic AL amyloidosis has not been systematically determined. On the one hand, there are well defined diagnostic criteria, it has a high mortality and short survival,[26] whilst on the other

hand, it may be misdiagnosed[44] and survival has steadily been improving,[213] introducing uncertainty into calculations of disease incidence. In this study, amyloidosis was present on the death certificates of 79% of individuals with known systemic amyloidosis in England and 82.5% of individuals with known systemic AL amyloidosis. Using the information from death certificates, an estimated 0.58/1000 deaths in England were attributable to amyloidosis, with a significant increase reported over the decade. Among those reviewed at the NAC who died, 80% had AL amyloidosis. It is estimated therefore that ~0.46/1000 deaths in England are attributable to AL amyloidosis. Patient survival increased substantially between 2000 and 2008 meaning that estimates of disease incidence calculated from death certificate data alone may be invalid.

Individual level data for patients reviewed at the NAC are likely to be extremely reliable particularly with respect to presence and type of amyloid. Only 48.2% of patients with systemic amyloidosis who died in 2008 had been seen at the NAC however, clearly suggesting that not all patients with systemic amyloidosis in England are seen at the centre. An estimate of incidence of systemic amyloidosis which is calculated solely on the basis of NAC cases will undoubtedly be an underestimate; however, 0.51 per 100000 population in 2008 can reliably be considered a minimum disease incidence. The true incidence of systemic amyloidosis is likely to be approximately double this figure, ~1.0 per 100000, although once again, this may be an underestimate since 21% of patients with systemic amyloidosis do not have amyloidosis on their death certificates. This estimate (10 per million population) is consistent with the reported incidence from Kyle *et al* of 8.9 per million person years.

The incidence, or perhaps more likely, recognition of systemic amyloidosis appears to be increasing. Data from Olmstead County showed an apparent rise in incidence of AL amyloidosis during the last decade of their analyses. In the present study, there was a significant rise each year in the number of individuals with amyloidosis on their death certificate, accompanied by a parallel rise in the number of patients with systemic amyloidosis assessed at the NAC throughout the study period. This apparent rise may reflect an actual increase in disease incidence, but could equally well be contributed to through better awareness of the disease itself and/or existence of a national referral centre, as well as improved diagnostic techniques.

The most rapidly advancing area for diagnostic imaging is in cardiology. Increasing numbers of patients are diagnosed through echocardiography and cardiac MRI, and this may have a significant impact on disease incidence. This theme is explored further in the next chapter which is focussed on patients with isolated cardiac disease.

Study Limitations

From a combination of NAC data and death certificate data one can robustly calculate the sensitivity of death certificates in reporting systemic amyloidosis. Unfortunately the specificity of death certificate data for reporting systemic amyloidosis cannot be calculated since further clinical information is not available on many of the relevant individuals, although since amyloid histology is required for diagnosis and is extremely specific. It is therefore assumed that specificity is likely to be very high. The number of deaths among amyloidosis patients who were not seen at the centre has been used to make an estimate of the total number

of patients with amyloidosis in England. However, the proportion of patients who are diagnosed with amyloidosis but never seen at the NAC that have amyloidosis on their death certificates is not known. It is conceivable that patients with systemic amyloidosis who attend the NAC are more likely to have amyloidosis on their death certificate than those with systemic amyloidosis who do not attend the NAC.

Results Section Two: Cardiac Amyloidosis

Chapter Four: Senile Systemic Amyloidosis, Clinical Features at Presentation and Outcome

Introduction

ATTRwt amyloidosis is almost certainly under diagnosed[216] and as the prevalence increases with age and the population demographic shifts towards the elderly, the burden of disease will become an increasing problem.[61, 62] The results of chapter 3 showed an estimated incidence of ATTRwt in people over 80 years old to be 0.3 per hundred thousand population. In recent years sophisticated imaging techniques such as cardiac MRI have become increasingly used. This has resulted in higher detection rates of cardiac amyloidosis. The aging population and increased detection rate are likely to have a significant impact on the disease epidemiology and this is explored further in chapter 4.

Without effective treatment cardiac AL amyloidosis is a fatal disease. Correctly identifying the amyloid type is vital as it has a major impact on prognosis and completely dictates treatment. Biopsy followed by Congo Red staining and immunohistochemistry is the gold standard for diagnosing and typing amyloid.[200] Correct diagnosis of isolated cardiac amyloidosis clinically is especially challenging as symptoms are often non specific. The diagnosis of ATTRm is relatively straightforward if genetic screening of the TTR gene is performed but differentiating between ATTRwt and AL amyloidosis can be more problematic. ATTRwt invariably affects older patients who may well have an incidental MGUS. If the co-existence of amyloidosis and a plasma cell dyscrasia alone is

used as evidence of AL amyloidosis, chemotherapy based treatment may be given in error.[44]

Detection of late gadolinium enhancement on cardiac MRI is reported to be characteristic of amyloidosis. Some features appear more characteristic of ATTR amyloid such as transmural late gadolinium enhancement (LGE) and right ventricular LGE, but currently MRI appearances cannot differentiate between amyloid types.[215]

Currently there is no specific licensed treatment for ATTRwt amyloidosis, but new drugs are on the horizon with several clinical trials currently recruiting patients in transthyretin amyloidosis: http://clinicaltrials.gov/ct2/results?term=transthyretin.

Defining the clinical course of the disease is important both for patient counselling in a disease for which there is currently limited literature and to identify clinical diagnostic and response criteria for further treatment trials.[70,144]

The aims of this study were: firstly to characterise the disease natural history and identify factors which predict survival, and secondly to decipher whether baseline patient characteristics could be used to confidently diagnose the amyloid type thereby avoiding the need for cardiac biopsy.

Methods

Patients

One hundred and two patients with biopsy confirmed ATTRwt were analysed. Cases were diagnosed between 2002 and August 2011 at the NAC and compared to 36 patients with biopsy confirmed isolated cardiac AL amyloidosis diagnosed over the same time period.

In the AL group, 20 patients were referred from cardiology and 16 patients were referred by haematologists, all AL patients had been seen by a cardiologist prior to initial assessment. In the ATTRwt group, 73 patients were referred from cardiology, 18 from haematology, 3 from general practice, 2 each from gastroenterology and urology and one each from elderly care, neurology, rheumatology and respiratory medicine. Ninety three of the 102 patients in the ATTRwt group had been seen prior to referral by a cardiologist.

All patients had an initial diagnostic assessment. Onset of symptoms was defined on the basis of the history but a diagnosis of amyloidosis was dated from the biopsy, which provided histological proof of amyloid. Patients were then reviewed at 6-12 month intervals. Evidence of a plasma cell dyscrasia was defined as any of the following: detectable M-protein on plasma electrophoresis or immunofixation, Bence Jones proteins in urine or abnormal serum free light chain level (Kappa >19.4, Lambda >26.3) or abnormal ratio (0.26-1.65).

Diagnostic Procedures

Amyloid deposition was confirmed histologically in all patients.[200] All ATTRwt patients had immunohistochemical confirmation of transthyretin amyloidosis from a variety of tissues. All patients with transthyretin amyloidosis were confirmed to be wild type on genotyping.[208]

All patients had amyloidotic cardiomyopathy defined by either endomyocardial biopsy proof of amyloid deposition or echocardiographic features consistent with amyloid deposition.[60] Significant systemic amyloidosis was excluded by radiolabelled ^{123}I-labelled SAP scitnigraphy.[51]

Instrumental Definitions

ECG measures were based on standard definitions.[217] Echocardiographic measures of chamber quantification were based on standard recommendations.[218] In the survival analyses left ventricular wall thickness was graded as: mild 1.3-1.5cm, moderate 1.6-1.9cm and severe >2.0cm. Ejection fraction was graded as: normal >55%, mild impairment 45-54%, moderate impairment 36-44% and severe impairment <35%.

Classification of diastolic dysfunction on echocardiography was based on published data.[219] Not all patients were included in the classification of diastolic dysfunction due to lack of measured parameters in patients diagnosed before 2005. Patients who had AF with a rapid ventricular rate were excluded from grading of diastolic function. Patients with rate controlled AF were included when a combination of E/E', MVdecT and IVRT were measured.

Statistical Analysis

Results are expressed as mean (±SD), median (IQR) or percentage as appropriate. Patient follow-up was censored at the last clinic visit. Univariate analysis comparing the baseline data in the two groups used the unpaired *t* test (if the data were normally distributed) and the non-parametric Mann Whitney test for numerical variables, and Chi-square or Fisher's exact test for categorical variables. Factors that achieved statistical significance ($p<0.05$) and deemed clinically relevant were included in a multivariable logistic regression model (SPSS Statistics Version 19).

Patient survival was estimated by Kaplan-Meier analysis (Stata version 11: StataCorp LP). The log-rank test was used to compare differences in stratified Kaplan-Meier survival curves. Cox regression analysis using a backward stepwise approach was used to investigate factors associated with overall survival in ATTRwt patients, using IBM SPSS Statistics (Version 19) (Statistical significance was achieved if $p < 0.05$).

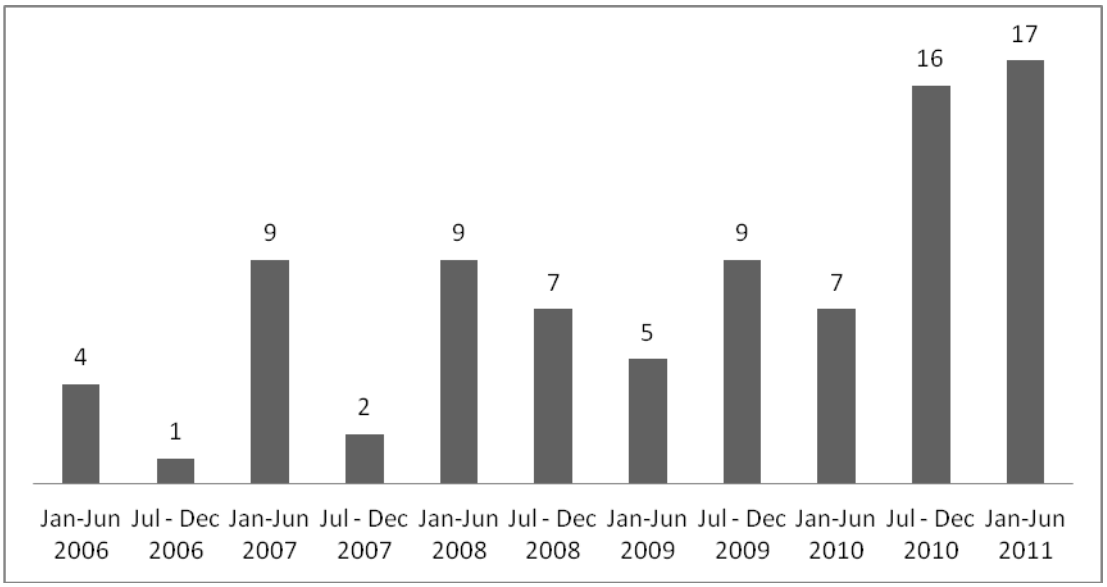
Results

Referral Patterns and Diagnosis

Of the 102 patients diagnosed with ATTRwt: 65 (63.7%) were diagnosed on endomyocardial biopsy, 24 (23.5%) on gastrointestinal biopsy, four (3.9%) from bladder biopsy, four (3.9%) on fat biopsy, one (0.9%) on carpal tunnel biopsy and one (0.9%) from fallopian tube tissue. Fifteen (41.6%) patients had amyloid proven on a biopsy prior to the initial assessment in the AL group and 46 (46.5%) in the ATTRwt group. The initial investigation which prompted the referral or biopsy in the AL group was: echocardiogram in 22 (61.1), CMR in 12 (33.35), one case each for bone marrow and CT scan. In the ATTRwt group the initial investigation which prompted referral or biopsy was echocardiogram in 55 (55.5%), CMR in 37 (37.4%), cystoscopy in 2 cases and one case each for colonoscopy, gastroscopy and cardiomegaly on chest x-ray. Two cases were referred on the basis of evidence of a plasma cell dyscrasia and a history of breathlessness. The 36 out of a total of 1953 patients with AL amyloidosis diagnosed with isolated cardiac AL amyloidosis after comprehensive evaluation, represented 1.84% of AL patients.

The number of diagnoses of ATTRwt has increased in the past five years with a doubling in the last 12 months. From 2007 to 2011 75-80% of referrals were from cardiologists each year.

Figure 4.1 Patients diagnosed with biopsy proven ATTRwt amyloidosis since 2006



Features in the History

Three patients did not have evidence of cardiac amyloid on echocardiography. These were patients with amyloid in tissue from gastrointestinal tract, bladder and carpal tunnel; these patients were therefore removed from further analyses.

Baseline (initial assessment) patient demographics of both groups is shown in Table 4.1. ATTRwt is a predominantly male disease; 88.8% of affected patients were men, significantly higher than the 69.4% of cardiac AL patients. The median age at presentation was 73 years, significantly older than 63 yrs in cardiac AL. Presenting symptoms were similar in both groups with the majority reporting breathlessness. The severity however was greater in the AL group with more patients describing NYHA class III/IV symptoms and over 50% of ATTRwt patients describing NHYA class I/II symptoms. The variety of presenting symptoms was much wider in the ATTRwt group than the AL patients. A history of arrhythmia was common, 43.4% had a history of AF which was the presenting

complaint in 10% of patients. AF was more common in the ATTRwt group than the AL patients. Eight percent of ATTRwt patients were found to have amyloid incidentally on routine testing or at the time of an operation for a different complaint, this feature was not seen in AL amyloidosis. TTR patients diagnosed by cardiac biopsy as opposed to other tissues were younger (median age at diagnosis of 72 vs. 77yrs (P=<0.01)) with a lower NT-proBNP (median 295pmol/L vs. 540pmol/L, P=0.03). Although there was a higher mitral valve E/A ratio (median 2.7 vs. 1.6, P=0.01)) in the cardiac biopsy group, all other measures of diastolic dysfunction (IVRT, E/E' and mitral valve deceleration time) were not significantly different, implying no real difference in overall diastolic function.

A history of carpal tunnel syndrome was significantly more common in the ATTRwt group than the AL group (48.5% vs. 8.3%). In the AL group 2 patients had symptoms of carpal tunnel at the time of diagnosis and one had a release a year prior to diagnosis. In the ATTRwt group 12 patients had symptoms of carpal tunnel syndrome at the time of diagnosis and 40 had previous release operations a median of 8 (IQR 3-10) years prior to diagnosis.

Baseline Biochemical Evaluation

On univariate analysis (Table 4.1) there were no major differences in biochemical markers of liver function consistent with the SAP scans which showed no cases of hepatic amyloid deposits in either group. There was significantly more proteinuria in the AL group perhaps due to renal amyloid involvement which was below the threshold of detection by SAP scintigraphy. Four AL patients had >1g of proteinuria. Of these patients, one had diabetes and one had a duplex kidney, the remaining two did not have any comorbidity which would explain proteinuria.

There was no difference in eGFR in patients with proteinuria compared to those without. Three patients with ATTRwt had underlying renal diseases associated with >1g of proteinuria, all other patients had <1g of proteinuria at baseline. Three ATTRwt patients had underlying diseases such as longstanding hypertension and diabetes which could explain proteinuria; however the cause for low level proteinuria was not explained by co-morbidities in the other patients.

The most significant difference on univariate analysis was in NT pro-BNP measurements. This was a median of 714pmol/L in the AL group and 317.5pmol/L in ATTRwt group (P<0.001). Evidence of a plasma cell dyscrasia was found in all patients with cardiac AL but also as an incidental finding in 24.1% of patients with ATTRwt (P<0.001).

Baseline Cardiac Investigations

On univariate analysis of ECG features, significantly more patients were in AF or atrial flutter in the ATTRwt group and more patients had AV conduction abnormalities (Table 4.2). Low QRS complexes were only seen in 27.3% of the AL patients and 12.9% of ATTRwt patients. There were significant differences in several echocardiographic features between the two groups (Table 4.3). On univariate analysis patients with ATTRwt had significantly thicker walled hearts than the AL group. Both groups had predominantly diastolic dysfunction, most commonly grade III/IV, indicating a restrictive filling pattern. The E/E' was higher in the AL group (median (IQR) 21.76 (15.7, 26.06) vs. 15.81 (12.37, 17.91); P = <0.001). The mitral valve deceleration time was higher in the ATTRwt group (mean; 191.2 ± 59.35 vs. 147.9 ± 42.46; P = <0.001, CI 20.42-

66.26) and the IVRT was also higher in the ATTRwt group (mean ± SD 87.21 ± 25.41 vs. 74.35 ± 20.74; P = 0.01, CI 2.68-23.03). Median left ventricular ejection fraction was 47% in both groups.

Table 4.1 Baseline Patient Characteristics in Patients with ATTRwt and Isolated Cardiac AL Amyloidosis (*Either a detectable paraprotein or an abnormal free light chain ratio).

	AL (N= 36)	**ATTRwt (N=99)**	**P value**
Male, n (%)	25 (69.4)	88 (88.8)	0.01
Ethnicity, n (%)			
Caucasian –Northern European	33 (92)	92 (93)	0.36
Caucasian – Southern European	0 (0)	2 (2)	
North American	0 (0)	1 (1)	
Indian Subcontinent	1 (3)	0 (0)	
Sub-Saharan African	2 (5)	1 (1)	
Afro-Caribbean	0 (0)	3 (3)	
Age at diagnosis, years (IQR)	63.0 (56.6, 65.8)	73.0 (69.5, 78.2)	<0.001
Age at symptom onset , years (IQR)	60.2 (53.8, 65.2)	70.9 (67.7, 74.1)	<0.001
Age at death (IQR)	63 (56, 67)	77 (74, 81)	<0.001
NT pro-BNP, pmol/L (IQR)	714.0 (427.5,1573.0)	317.5 (212.3, 909.3)	<0.001
NT pro-BNP (age 70 and under), pmol/L (IQR)	633 (412, 1073)	293 (227, 404)	
NT pro-BNP (age over 70), pmol/L (IQR)	2127 (1498, 2755)	440 (244, 794)	
Troponin T, ng/mL (IQR)	0.05 (0.02, 0.1)	0.04 (0.02, 0.05)	0.3
Positive Troponin T, N (%)	19 (52.7)	51 (51.5)	
Hb, g/dL (IQR)	13 (12.4, 13.6)	14.8 (12.8, 14.8)	0.005
Albumin, g/L (IQR)	41 (39, 43)	44 (41.3, 46.0)	<0.001
Bilirubin, µmol/L (IQR)	14 (11, 24)	15 (13, 28)	0.2
ALP, U/L (IQR)	95 (72, 148)	106 (77, 138)	0.5
GGT, U/L (IQR)	75.5 (41.0, 142.3)	111.0 (71.0, 162.3)	0.1
eGFR, ml/min (IQR)	64 (48, 87)	63 (41, 69)	0.2
24 hour urine protein, g (IQR)	0.39 (0.1, 0.8)	0.1 (0.1, 0.2)	<0.001
Lambda free light chain, mg/L (IQR)	255 (116, 468)	16 (13, 21)	<0.001
Missing, n (%)	0 (0)	8 (8)	
Kappa free light chain, mg/L	31 (7, 22)	18 (14, 24)	0.01
Missing, n (%)	0 (0)	8 (8)	
Detectable paraprotein (%)	24 (66)	14/93 (15)	<0.001
Missing, n (%)	0 (0)	6 (6)	
Any detectable plasma cell dyscrasia* n (%)	36 (100)	22/91 (24%)	<0.001
Missing, n (%)	0 (0)	8 (8)	
Supine systolic Blood Pressure, mm Hg (IQR)	107 (95, 118)	116, (107, 134)	0.006
Supine diastolic Blood Pressure, mm Hg (IQR)	72 (60, 76)	74 (66, 81)	0.1
Orthostatic hypotension, n (%)	7 (19)	9 (9)	0.1
Primary presenting symptom, n (%)			
Breathlessness	29 (80.5)	53 (53.5)	
Atrial fibrillation/Flutter	0 (0)	10 (10)	
Oedema	0 (0)	8 (8)	
Incidental finding	0 (0)	8 (8)	
Syncope	1 (3)	6 (6)	
Palpitations	0 (0)	3 (3)	
Chest pain	2 (5.5)	3 (3)	
Orthostatic hypotension	0 (0)	2 (2)	
Frank haematuria	0 (0)	2 (2)	
Carpal tunnel syndrome	0 (0)	1 (1)	
Cough	0 (0)	1 (1)	
Diarrhoea	0 (0)	1 (1)	
Dizziness	0 (0)	1 (1)	
Chest sepsis	1 (3)	0 (0)	
Lethargy	3 (8)	0 (0)	
NYHA Class, n (%)			
I	0 (0)	35 (35)	<0.001
II	14 (38)	26 (26)	
III	17 (47)	25 (25)	
IV	5 (13)	6 (6)	
missing	0 (0)	7 (7)	
Weight loss, n (%)	14 (38)	18 (18)	0.02
History of atrial fibrillation, n (%)	6 (16)	43 (43)	0.004
History of ischaemic heart disease, n (%)	4 (11)	27 (27)	0.06
Pacemaker, n (%)	2 (5.5)	13 (13)	0.4
Previous Normal Coronary Angiogram, n (%)	8 (22)	12 (12)	0.2
Previous Coronary Artery Bypass Graft, n (%)	0 (0)	8 (8)	0.1
History of chest pain, n (%)	3 (8)	14 (14)	0.6
History of carpal tunnel syndrome, n (%)	3 (8)	48 (48)	<0.01
History of lower limb neuropathy, n (%)	3 (8)	9 (9)	1.00
Macroglossia or bruising, n (%)	9 (25)	0 (0)	<0.001

Table 4.2 Baseline Electrocardiographic Features in patients with ATTRwt and Isolated Cardiac AL amyloidosis (*Percentage of patients who were not paced are displayed)

	AL (N = 34)	**ATTRwt (N= 93)**	**P value**
Rhythm, n (%)			
Sinus	28 (82)	42 (45)	0.002
Atrial Fibrillation	4 (11)	36 (38)	
Atrial Flutter	0 (0)	7 (7.5)	
Paced	2 (5)	8 (8)	
Mean voltage leads II/III/AVF (mm)	5.4 ± 3.6	2.9 ± 2.9	0.4
Total QRS score leads II/III/AVF (mm)	14.9 ±10.2	17.5±8.6	0.1
Mean voltage leads V4/V5/V6 (mm)	11.5 ± 5.5	13.5 ± 4.7	0.2
Total QRS score leads V4/V5/V6 (mm)	30.7± 10.9	39.6±14.9	0.01
Low QRS complexes* (N/%)	9 (27)	11 (13)	0.2
Any AV conduction abnormality* n (%)	14 (43)	50 (58)	0.2
Right Bundle Branch Block	1 (3)	14 (16)	
Left Bundle branch block	2 (6)	17 (20)	
First degree heart block	5 (15)	10 (11)	
Bi fascicular block	5 (15)	9 (10.5)	
Junctional rhythm	1 (3)	0 (0)	
QT interval (ms)	401 ± 71.5	430.1 ± 55.2	0.003
QTcB (ms)	596.6 ± 745.0	478.7 ± 53.3	0.2
T wave inversion, n (%)	18 (53)	37 (39)	0.2

Table 4.3 Baseline Echocardiographic Parameters in Patients with ATTRwt and Isolated Cardiac AL Amyloidosis

	AL	**ATTRwt**	**P value**
Interventricular septal thickness in diastole (IVSd), (cm)	1.5 ± 0.2 (n = 34)	1.7 ± 0.3 (n = 95)	<0.001
Left ventricular posterior wall thickness in diastole (LVPWd), (cm)	1.5 ± 0.2 (n = 34)	1.7 ± 0.2 (n = 95)	<0.001
Left ventricular internal dimension in diastole (LVIDd) (cm)	4.2 ± 0.4 (n = 34)	4.4 ± 0.6 (n = 95)	0.1
LV Ejection Fraction (%)	47.8 ± 12.6 (n = 34)	46.6 ± 12.8 (n = 95)	0.9
E/A Ratio	2.6 ± 1.3 (n = 26)	2.4 ± 1.0 (n = 66)	0.9
E/E^{I} (IQR)	21.8 (15.7, 26.1) (n = 31)	15.8 (12.4, 17.9) (n = 86)	<0.001
Mitral valve deceleration time (MVdecT) (ms)	147.9 ± 42.5 (n = 31)	191.2 ± 59.4 (n = 91)	<0.001
Iso-volumetric relaxation time (IVRT) (ms)	74.4 ± 20.7 (n = 31)	87.2 ± 25.4 (n = 79)	0.01
Tissue Doppler imaging lateral wall in diastole (TDIs wave lateral) (ms)	0.06 ± 0.02 (n = 20)	0.06 ± 0.05 (n = 70)	0.4
Grade of diastolic dysfunction, n (%)	(n = 29)	(n = 76)	
Normal	0		0.4
I	2 (7)	10 (13)	
II	5 (17)	19 (25)	
III/IV	22 (76)	47 (62)	

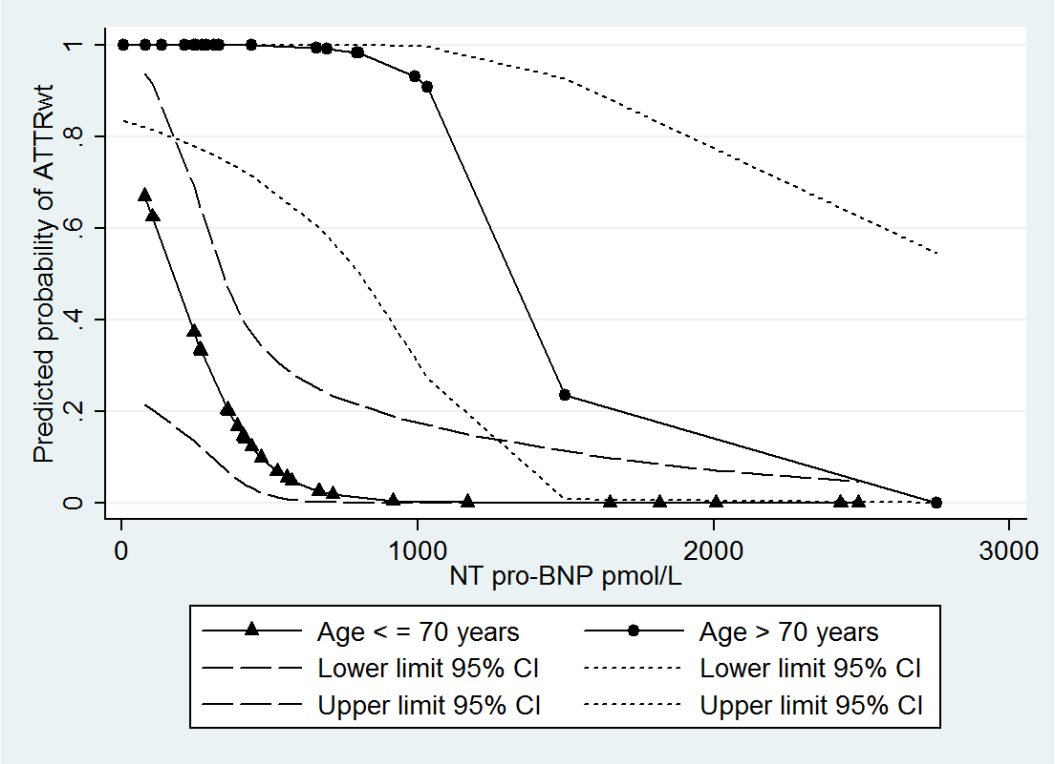
(*Due to varying times at which echocardiography was performed not all measures were reported in all patients).

Distinguishing Between Patients with Isolated AL Amyloidosis and ATTRwt

Baseline characteristics which could distinguish between the two diagnoses were identified. All patients in the AL amyloidosis group had a detectable underlying plasma cell dyscrasia, indicating that if no plasma cell dyscrasia could be identified in this cohort, the diagnosis was ATTRwt. No patient with ATTRwt had evidence of macroglossia which was purely an indicator of AL amyloidosis. A logistic regression model was used on the remaining 47 patients (25 patients with AL amyloidosis and 22 patients with ATTRwt) who had a detectable plasma cell dyscrasia and no macroglossia. There were no clinically relevant differences in baseline characteristics in the excluded patients when stratified by amyloid type. NT pro-BNP and the age at diagnosis were both associated with risk of ATTRwt. NT pro-BNP was higher in patients with AL amyloidosis. The odds of having ATTRwt reduced by 0.1% for every pmol/L unit increase in NT-pro BNP (P=0.04). Likewise patients with ATTRwt were older, the odds of ATTRwt increasing by 102% for every 1 year increase in age (P=0.02). A further logistic regression analysis which replaced actual age at diagnosis with age at diagnosis as a binary variable (27 patients aged $\leq$ 70 years and 20 patients aged > 70 years) indicated that both the binary age variable and NT pro-BNP at baseline were significantly associated with diagnosis (P=0.009 and P=0.03, respectively). Figure 4.2 shows the probability of having ATTRwt amyloidosis in patients who have isolated cardiac amyloidosis without macroglossia or easy bruising, with a detectable plasma cell dyscrasia in patients above or below 70 years of age, according to the NT-proBNP at baseline. Patients less than or equal to 70 years

old were more likely (probability > 0.5) to have ATTRwt than AL if the NT pro-BNP was <183pmol/L and patients over 70 years old with an NT-proBNP of <1420pmol/L were more likely to have ATTRwt. Using this combination of age and NT-proBNP, the positive predictive value was 90.0% and negative predictive value, 85.2%, with a positive likelihood ratio of 10.2 and negative likelihood ratio of 0.19. The area under the ROC curve was 0.98 (CI 0.95-1.0), sensitivity 83.3% and specificity 92%.

Figure 4.2 Predicted probability of wild type transthyretin amyloidosis in patients aged 70 years and below or over 70 years with a detectable plasma cell dyscrasia by NT pro-BNP.



Patient Survival

Survival from diagnosis in patients with ATTRwt was significantly longer than for the cardiac isolated AL group (log rank test P=0.001). Median survival for patients with ATTRwt was 2.71 years and in cardiac AL amyloidosis survival was 0.87 years (Figure 4.3). Median survival from the onset of symptoms was much longer than from diagnosis in the ATTRwt group; 6.07 years compared to 1.7 years in the cardiac AL group (log rank test P=<0.0001 (Figure 4.4). There was no difference in survival in ATTRwt patients with or without a detectable clone.

A Cox proportional hazards multivariable survival analysis of the association of variables at diagnosis and death in patients with ATTRwt amyloidosis is shown in Table 4.4. A positive troponin T was strongly associated with death; hazard ratio (HR) 4.99 (95% CI 2.05-12.13: P=<0.001), as was having a pacemaker *in situ*; HR 4.90 (95% CI 1.74-13.80: P=0.003). There was no significant difference in survival between patients with NYHA class II/ III symptoms and those with class I. NYHA class IV symptoms were however associated with a significantly higher risk of death, HR 15.14 (95% CI 3.59-63.80: P=<0.001). However the confidence limits are wide for this variable. Baseline factors entered into the model which showed no significant association with death were; age, NT-proBNP per unit increase, IVSd graded as mild, moderate or severe, grade of diastolic dysfunction, ejection fraction graded as normal, mild, moderate or severe, AV conduction abnormality or atrial fibrillation on ECG.

Thirty two patients died in the ATTRwt group. The primary cause of death reported on the death certificates were as follows; 17 from congestive cardiac failure, and one each of: complete heart block, hypertensive heart disease, ischaemic heart disease, ischaemic stroke, old age, multi-organ failure, renal

failure, acute myeloid leukaemia, mesothelioma, metastatic lung cancer, urinary tract infection and pneumonia. The cause of death is unknown to us in three patients. Only two patients in the AL amyloidosis group developed extra-cardiac amyloid, one patient had symptoms of progressive peripheral neuropathy and one patient developed end stage renal failure. Twenty one patients (58%) died before their 6 month follow-up appointment.

Table 4.4 Cox Regression model of survival outcome from diagnosis in patients with ATTRwt amyloidosis.

Variable	**N**	**Hazard Ratio**	**95% CI**	**P value**
Positive Troponin T	51	4.9	2.1-12.1	< 0.001
Pacemaker	13	4.9	1.7-13.8	0.003
NYHA Class				
I	35	Reference		
II	26	2.5	0.8-2.5	0.14
III	25	1.8	0.6-1.8	0.29
IV	6	15.1	3.6-63.8	<0.001
IVSd (cm)				
1.3-1.5	19	Reference		
1.6-1.9	52	0.4	0.2-1.1	0.09
>2.0	15	0.4	0.1-1.1	0.08

Figure 4.3 Patient survival from diagnostic biopsy**.** Median survival in patients with wild type transthyretin amyloidosis from diagnostic biopsy is 2.71 years compared to 0.87 years in patients with isolated cardiac AL amyloidosis. Overall survival is significantly longer in the ATTRwt group (P=0.002 Log-rank (Mantel-Cox) Test).

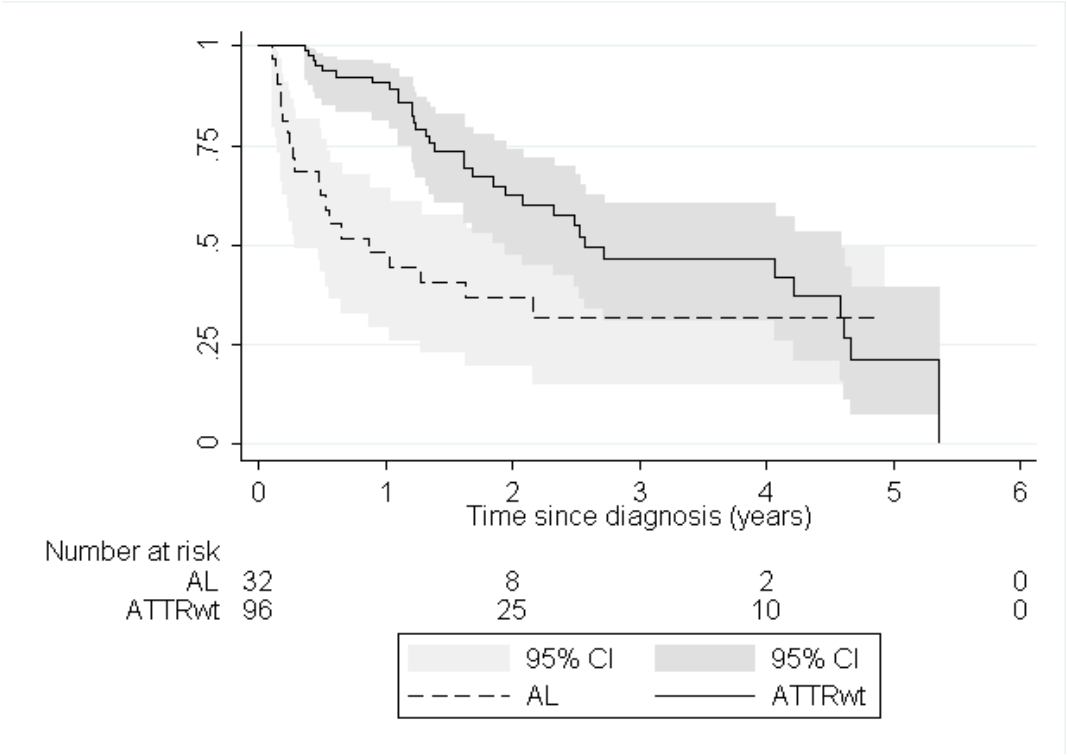
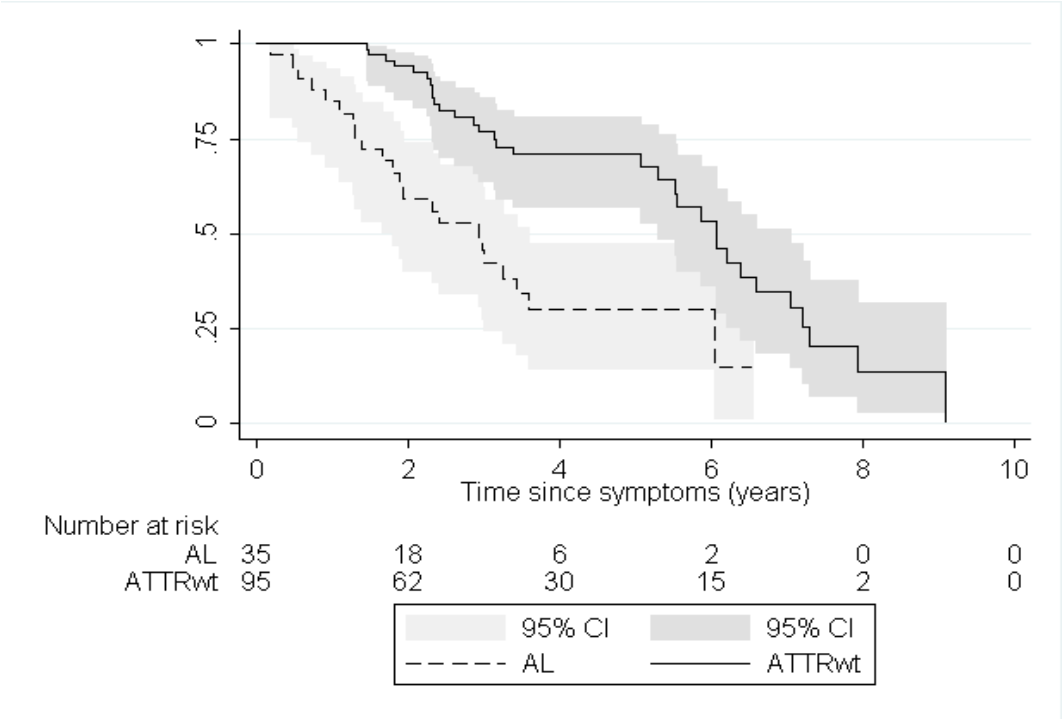


Figure 4.4 Patient survival from onset of symptoms. Median survival in patients with wild type transthyretin amyloidosis from onset of symptoms is 6.07 years compared to 1.7 years in patients with isolated cardiac AL amyloidosis. Overall survival from symptoms is significantly longer in the ATTRwt group (P=<0.0001 Log-rank (Mantel-Cox) Test).



Discussion

ATTRwt amyloidosis is a slowly progressive disease of the elderly whose true incidence is unknown. ATTRwt amyloid has been shown to deposit in the carpal tunnel.[142] A recent series has reported ATTRwt deposits in 34% of patients with idiopathic carpal tunnel syndrome.[220] Connors *et al* recently reported that carpal tunnel syndrome was rare in patients with ATTRwt cardiac amyloid.[221] It has been suggested that systemic deposition of TTR occurs years before the onset of cardiac dysfunction.[222, 223] In this series, 48% of ATTRwt patients gave a history of carpal tunnel syndrome which preceded the onset of clinical symptoms of heart failure in 77%, a median of 8 years prior to the diagnosis of amyloidosis being made, consistent with a slowly evolving disease. ATTRwt can be found in other tissues aside from the myocardium and wrist.[140, 224, 225] Almost 4% of patients attending the NAC presented with frank haematuria or an incidental finding on bladder histology. No patients have had amyloid detectable on radiolabelled ^{123}I SAP scintigraphy.

The apparent increase in the disease over the last couple of years reflects an increase in the number of referrals to the NAC. Whilst the total number of amyloid referrals has risen the proportion referred from cardiologists has vastly increased. Thirty seven percent of cases had amyloid suspected on the basis of CMR findings. The use of sophisticated cardiac imaging techniques such as CMR in elderly patients are now more readily available and as the population ages the prevalence of the disease may well be increasing.

Studies describing the natural history of the disease have been limited to small numbers.[70] The largest study to date was reported this year by Rapezzi et al, it describes the long term follow-up of 186 patients with ATTRm and compares

them to 30 ATTRwt controls.[267] This chapter described the long term follow-up of 102 patients with ATTRwt amyloidosis. Patients predominantly presented with heart failure, although breathlessness was generally less severe than in cardiac AL amyloidosis despite greater LV wall thickness. Patients with ATTRwt amyloidosis had more arrhythmias than those with AL and were more likely to have required pacing before diagnosis. These findings are consistent with those reported by Rapezzi *et al* where patients with ATTRwt were found to have greater LV wall thicknesses than those with cardiac AL and a higher incidence of left bundle branch block.[70] This area will be further explored in the next chapter which uses holter monitors to identify the prevalence of dysrhythmias in cardiac amyloidosis.

Low QRS complexes are widely considered a marker of diagnosis of cardiac amyloidosis. In this study the prevalence of this is lower than previously described.[226] Whilst low voltage QRS complexes on the ECG remain a potential indicator of cardiac amyloid, it is important to note that normal size complexes should not deter physicians from considering a diagnosis of amyloid.

A marked survival difference between patients with ATTRwt and cardiac AL amyloidosis has been reported before.[70, 221] Median survival in patients with ATTRwt was 2.71 years from diagnosis compared with 0.87 years in the AL group. This is even more striking when survival from symptom onset is considered; the median survival in the ATTRwt group was 6.07 years and this may be a more useful indicator for patients who have been picked up early on in the course of the disease or whose cardiac amyloid is an incidental finding.

The presence of a pacemaker was associated with a nearly 5 fold increase risk of death by Cox regression. Clearly this does not necessarily imply that pacemaker

insertion is a bad idea; indeed it seems likely that patients with conduction abnormalities necessitating a pacemaker are at higher risk of sudden cardiac death. A recent study in FAP reported that prophylactic pacemakers prevented major cardiac events in 25% of patients.[227] There is no specific literature on the role of implantable cardiac defibrillators in ATTRwt amyloidosis but in AL type it seems that very careful patient selection is required as most sudden cardiac death is reportedly due to pulseless electrical activity which is not amenable to cardioversion.[228]

A positive troponin T at baseline was associated with an almost 5 fold increased risk of death. Suhr *et al* reported NT pro-BNP to be a sensitive marker for diagnosing ATTR cardiomyopathy in hereditary Val30Met patients.[229] Recent data from Russo *et al* suggested that both troponin and BNP are prognostic indicators in ATTR amyloidosis. Troponin appeared to be a stronger predictor (HR 2.2 95% CI 1.4-3.5, p=<0.01) compared to BNP (HR 1.2 95% CI 1.1-1.4, P<0.01).[230] Interestingly higher baseline NT pro-BNP in our cohort was not associated with death in ATTRwt. Functional impairment was only of significance at NYHA class IV symptoms which conferred a HR of death of 15.14, however the confidence limits for this variable were wide indicating that this may not be a truly significant result. Although LV wall thickness has been reported to be associated with survival in patients with cardiac amyloidosis,[70] it is not clear that this is the case in ATTRwt as these patients were a very small proportion and not separately analysed in the current published literature. In this study echocardiographic features such as wall thickness and degree of diastolic or systolic dysfunction did not predict survival.

The combination of transthyretin amyloid on endomyocardial biopsy and wild type TTR gene sequencing is the gold standard for diagnosing ATTRwt. Whilst endomyocardial biopsy carries a low reported risk of complications of between 1-2%,[231] some elderly patients are not keen to undergo such an invasive investigation. Tissue from screening biopsies such as abdominal fat and rectum can be helpful in diagnosing amyloid, however false negatives can occur due to patchy amyloid deposition and Connors *et al* recently reported that only 27% of fat aspirates in ATTRwt were positive.[221] When histological confirmation is not possible clinicians must rely on a combination of clinical history, imaging and biochemical markers to come to a diagnosis. 99mTc- DPD scintigraphy has shown to be a sensitive imaging technique in diagnosing cardiac transthyretin amyloidosis.[65, 232, 233] Studies are currently limited by small numbers but the technique appears to be highly specific for transthyretin amyloidosis[67] and it is emerging as a helpful diagnostic tool; however the availability of the technique is currently limited to centres within Europe.

The prevalence of MGUS in the general population is 5.3 percent among the over 70s, rising to 7.5 percent among those 85 years of age or older.[234] Twenty four percent of patients with proven ATTRwt in this series were found to have a detectable plasma cell dyscrasia. This incidence is higher than expected and reflects the increased detection rates of incidental low grade clones through the use of the free light assay. It is also likely that there is referral bias with amyloidosis sought most diligently in patients with a detectable clonal excess. It is unlikely that this high incidence of abnormal clones have confounded the phenothype. Survival was no different in the ATTRwt group when comparing those with or without a detectable clone; all patients have biopsy proof of amyloid

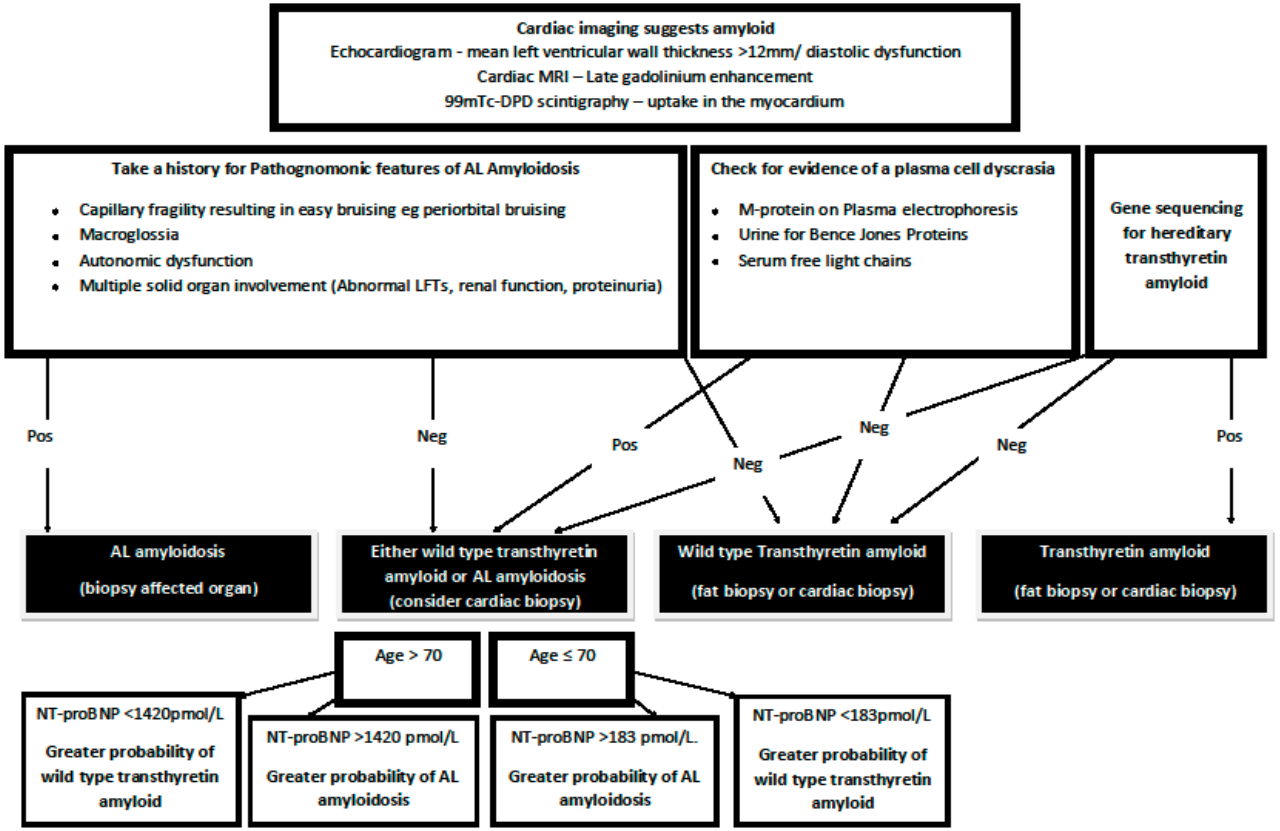
fibril type, and in all cases the clonal abnormality was very subtle, with no patient diagnosed with overt myeloma. The cause of death in the ATTRwt group did not appear to be related to the underlying clonal disease in any case.

The presence of a clone cannot be used to diagnose AL type amyloidosis without further tissue confirmation. Clearly the converse is also a concern although in this series all of the patients with isolated cardiac AL amyloidosis did have a detectable plasma cell dyscrasia. Indeed at the NAC cardiac biopsy proven isolated cardiac amyloidosis accounts for only 1.84% of patients with systemic AL amyloidosis. Although it has been reported that 6% of patients with AL amyloidosis have no detectable clone,[76] our recent experience from the prospective ALchemy cohort[77] suggests that only one of 494 new patients with AL amyloidosis seen at our centre had no evidence of a plasma cell clone on serum and urine investigations. One would therefore predict that it is extremely rare for a patient with AL amyloidosis to present with isolated cardiac disease and no detectable clone, although this is theoretically possible.

A much more frequent clinical problem is differentiating between ATTRwt and AL type in elderly patients who have isolated cardiac amyloid and an abnormal clone. Cardiac biopsy remains the only definitive method of distinction, and some patients are not prepared to undergo this invasive procedure. In this study, despite excluding patients with isolated cardiac amyloid and symptoms which are pathognomonic of AL amyloidosis such as macroglossia or easy bruising,[76] it was possible to estimate the risk of ATTRwt amyloid in patients with a detectable monoclonal protein by using a combination of age and NT pro-BNP. NT pro-BNP is dependent on factors such as renal function, arrhythmias and fluid balance. Although these factors all display co-linearity with NT pro-

BNP they were discarded on logistic regression analysis and NT pro-BNP remained a strong predictor of the diagnosis of either AL or ATTRwt assessment of a patient with amyloidosis. Figure 4.5 shows a flow diagram which outlines the diagnostic process in patients diagnosed with cardiac amyloid which uses age at diagnosis and level of NT-proBNP to help distinguish between ATTRwt and isolated cardiac AL amyloidosis in patients with a plasma cell dyscrasia.

Figure 4.5 Diagnostic flow diagram for patients presenting with suspected cardiac amyloidosis based on cardiac imaging. Initial investigations should include; a comprehensive patient history and examination; investigations for an abnormal clone and genotyping for hereditary transthyretin amyloidosis.



Chapter Five: Holter Monitoring in Systemic Amyloidosis

Introduction

Infiltration of the heart confers a poor prognosis in AL amyloidosis and is associated with sudden death.[123, 235] Patients with cardiac AL amyloidosis (cAL) frequently have conduction disturbances on ECG and indeed, ECG abnormalities may indicate cardiac involvement.[60] Previous small studies have described frequent complex ventricular arrhythmias in patients with AL amyloidosis.[236] There have been few studies in patients with cardiac transthyretin (cATTR) amyloidosis and data is scant. The results from chapter 4 showed that significantly more patients with isolated cardiac AL amyloidosis were in sinus rhythm on the ECG than those with ATTRwt (82% vs 45%, P = 0.02). Atrial fibrillation was common in patients with ATTRwt (38%), but syncope was rarely the presenting symptom in either disease. Of the thirty two patients who died in the ATTRwt cohort only one had complete heart block documented as the primary cause of death and no patients were described as dying from a sudden cardiac death or reported arrhythmia. These results could suggest that rhythm disturbances are common but the significance is relatively unknown in the ATTRwt phenotype. Data reported in patients with familial amyloid polyneuropathy show that patients frequently require pacemakers after liver transplantation suggesting that bradycardia and heart block may complicate cATTR.[227]

Histological analyses have shown that amyloid fibrils may have an affinity for the His-purkinje system, and prolongation of infra-His conduction times are common in cAL.[237,238] Median left ventricular wall thickness at the time of diagnosis is greater in cATTR patients than those with cAL.[226] One could therefore hypothesize that the greater cardiac mass could be associated with increased disruption of the His purkinje system and thus an increase in arrhythmic activity, however, the prognosis is much better in cATTR than in cAL.[70] This may indicate that arrhythmias are not the leading mode of death in these patients or that the pathological effects of these two amyloid fibril proteins are markedly different. There is limited data regarding the mode of death in patients with cardiac amyloidosis. Electromechanical dissociation was determined as the cause in the majority of AL patients in one study of prophylactic implantable defibrillators,[228] and based on this data, there has been a general reluctance to implant defibrillators into patients with cardiac amyloidosis.

The primary aims of chapter 5 are to determine the spectrum and frequency of rhythm disturbances in patients with cardiac amyloidosis and compare the two main amyloid types that affect the heart, AL and ATTR, and to determine whether there is an association between severity of cardiac amyloidosis by echocardiographic parameters and prevalence of dysrhythmias.

Methods

Patient Selection and Characterisation

This single UK centre prospective study was performed between May 2010 and June 2012. All patients newly referred to the NAC with suspected systemic amyloidosis were eligible for 24 hour Holter monitoring. Due to the limited availability of Holter equipment at the NAC, placement of monitors was prioritised to patients thought, on the basis of their referral letter, to have cardiac involvement by amyloid.

All study patients were asked to record periods of strenuous activity and symptoms during the monitoring period by means of a diary card (Appendix 1). During the clinical consultation, doctors were required to complete a detailed case report form (Appendix 2), which included recording information on: previous cardiac history, current symptoms of syncope, palpitations, chest pain, breathlessness, NYHA class, exercise tolerance and medications.

Investigation of all study patients included an electrocardiogram, echocardiogram and biochemical analysis of serum. Biochemical analysis included the cardiac biomarkers troponin T and NT-proBNP, and serum potassium, calcium and magnesium concentration as well as thyroid function tests. The assay for troponin T was replaced by a high sensitivity assay in November 2010. A positive Troponin T was defined as >0.03 ng/mL for the low sensitivity assay and >0.014 ug/L for the high sensitivity assay.

Diagnosis

Diagnosis of amyloidosis was based on histological proof of amyloid and was obtained from a variety of tissues, as previously described.[200] Genotyping was performed in patients who were suspected of having hereditary amyloidosis from their clinical assessment.[208, 239] Presence of cardiac amyloidosis was defined as a mean left ventricular wall thickness greater than 12 mm in the absence of hypertension or other potential causes of left ventricular hypertrophy, according to consensus criteria for AL amyloidosis.[60] The same criteria were applied to patients with ATTR amyloidosis to define presence of cardiac amyloidosis since, to date, separate criteria have not been defined.

Monitoring Procedures

Continuous twenty-four hour electrocardiographic monitoring (Holter monitoring) was performed using a portable cassette recorder with three lead placements (Spacelabs Healthcare Lifecard CF). Analysis of the 24 hour records was performed by an experienced electro physiologist using Pathfinder Digital Software V8.701 (Spacelabs). Two consultant cardiologists reviewed the traces to verify the reported results.

Ventricular arrhythmias were graded as previously described.[240] To recap briefly, Grade 0, no ventricular premature depolarisations; Grade 1, occasional ventricular premature depolarisations, no more than 30 per hour; Grade 2, more than 30 ventricular polarisations per hour; Grade 3, multiform ventricular depolarisations; Grade 4, couplets (two consecutive ventricular depolarisations); Grade 5, non sustained ventricular tachycardia (three or more consecutive ventricular beats at a rate of ≥120 beats/min, lasting for <30seconds).

Statistical Analysis

Patient survival was measured from the date of the Holter recording using Kaplan-Meier analysis. The Log-rank test was used to compare survival using the Graphpad prism software, version 5.

Continuous data were compared using a one-way analysis of variance (ANOVA) for parametric samples with Bonferroni post hoc analysis and Kruskal Wallis for non parametric variables. P values were adjusted for multiple testing accordingly. Differences in frequencies were compared using chi-square or Fisher's exact tests where appropriate using SPSS software, version 20. A P value <0.05 was considered significant.

Results

In total 452 patients had 24 hour Holter monitors performed between May 2010 and June 2012. During the recruitment period a total of 956 patients were reviewed at the NAC with suspected systemic amyloidosis including 192 with cAL and 137 with cATTR. Fifty eight of the 452 patients who had Holter monitoring were subsequently discovered not to have systemic amyloidosis and their data was not analysed as part of this study. The 394 remaining 'study patients' were censored on 13th December 2012, 6 months after recruitment of the last individual. Of 394 study patients, 156 had cAL, 94 had cATTR, and 144 had amyloidosis without cardiac involvement (non-cardiac). Patients with cATTR were more often male (84%) and were significantly older than those in the other two groups.

Baseline Characteristics

Baseline biochemical characteristics are shown in Table 5.1. There were statistically significant differences in potassium and Hb but these were not clinically significant. NT-proBNP was significantly higher in the cAL group compared to the cATTR group which was in turn higher than the non-cardiac group. The number of patients with a positive Troponin T was no different between the two cardiac groups but both cardiac groups had a greater proportion of patients with a positive Troponin T than the non-cardiac patients. Systolic blood pressure was higher among non-cardiac patients than those with cAL or cATTR.

Cardiac amyloidosis was defined according to consensus criteria which use echocardiography alone to define cardiac amyloidosis. In the non cardiac group a substantial proportion of patients had an elevated Troponin T and NT pro BNP (table 5.1). Fourteen patients (9.7%) had an NT-pro BNP greater than 332pMol/L (MAYO stage 2 or 3) of these 10 had an eGFR less than 30. Of those patients with a positive troponin 33 (45%) had an eGFR <60 which could explain the result. Thirty nine patients however had a positive troponin which could not be explained by renal disease suggesting that they may have cardiac amyloid which is below the threshold for detection by echocardiography.

Echocardiographic parameters varied between the three groups (Table 5.2), wall thickness was thickest in the cATTR patients followed by cAL. Systolic function characterised by EF was best in the non-cardiac patients and worst in the cATTR group. There was no difference in measures of diastolic function between the cAL and cATTR patients, but both cardiac groups showed significantly worse diastolic function than non-cardiac patients. Small complexes were seen on the ECG in 50% of cAL patients significantly more than the cATTR cohort (25.3%) (P<0.001). Small complexes were seen on the ECG in a significant proportion (30.4%) of patients in the non-cardiac group.

Table 5.3 shows the frequency of cardiac symptoms and cardiac medications. A previous history of arrhythmia was present in a significantly greater proportion of cATTR patients (53%) than cAL (23%) (P<0.001) and non-cardiac (13.5%)(P<0.001). Shortness of breath featured as a presenting symptom in 75.8% of cAL and 77.8% of cATTR patients, significantly more than non-cardiac patients (34.8%) (P<0.001). The severity of breathlessness measured by NHYA class was not different between the cAL and cATTR groups. In both

cardiac groups patients were described mostly as NYHA class II whilst the non-cardiac patients were mostly described as having NYHA class I symptoms (55.9%). The distribution of patients according to the MAYO staging system was no different in the two cardiac groups with most patients presenting as stage 3 disease.

Patients with cATTR were prescribed more anti-hypertensive medications than cAL and non-cardiac patients. Significantly more cATTR patients were taking B-blockers (cATTR 49.4% vs. cAL 28.7% ($P=0.002$) and non-cardiac 18.7% ($P<0.001$)).

Table 5.1 Baseline Biochemical Characteristics in Patients who had Holter Monitoring

	Overall P value	**AL Cardiac N = 156**	**Post Hoc P value Cardiac AL vs. Cardiac TTR**	**Cardiac TTR N = 94**	**Post Hoc P value Cardiac TTR vs. Non Cardiac**	**Non Cardiac N = 144**	**Post Hoc P value Cardiac AL vs. Non-cardiac**
Age, years (CI)	<0.001	64 (63, 66)	<0.001	74 (72, 75)	<0.001	67 (65, 68)	0.163
Sex male (%)	<0.001	103 (66)	0.006	79 (84)	<0.001	82 (57)	0.366
Ca, mmol/L (CI)	0.013	2.3 (2.26, 2.30)	0.017	2.2 (2.21, 2.25)	0.41	2.3 (2.25, 2.30)	0.99
K, mmol/L (CI)	0.006	4.4 (4.25, 4.45)		4.5 (4.39, 4.60)		4.6 (4.47, 4.66)	
Hb, g/dL (CI)	0.001	12.7 (12.43, 13.04)	0.02	13.4 (13.07, 13.73)	<0.001	12.4 (12.12, 12.75)	0.470
Mg mmol/L (CI)	0.062	0.83 (0.82, 0.85)		0.85 (0.83, 0.86)		0.82 (0.80, 0.84)	
Creatinine µmol/L (IQR)	0.266	99 (75, 150)		105 (88, 137)		96 (69, 153)	
eGFR, ml/min (CI)	0.879	60 (56.2, 64.46)		59 (55.14, 63.33)		60 (56.35, 65.55)	
TSH mU/L (CI)	0.064	3.4 (2.95, 3.86)		2.9 (2.29, 3.41)		2.7 (2.43, 3.07)	
NT pro-BNP, pMol/L (IQR)	<0.001	563 (222, 1271)	0.045	423 (222, 720)	<0.001	51(17, 113)	<0.001
Trop T positive N (%)	<0.001	125 (80)	0.21	84 (89)	<0.001	72 (50)	<0.001
Sys BP, mmHg (CI)	<0.001	114 (111, 117)	0.245	118 (115, 122)	<0.001	130 (127, 134)	<0.001
Dias BP, mmHg (CI)	0.003	70 (68, 72)	0.375	74 (70, 75)	0.411	75 (73, 77)	0.002
BMI, kg/m^2 (CI)	0.266	25 (25, 26)		25 (24, 26)		26 (25, 27)	

Table 5.2 Baseline Echocardiographic Parameters in Patients who had Holter Monitoring

	Overall P value	Cardiac AL	P value Cardiac AL vs. Cardiac TTR	Cardiac TTR	P value Cardiac TTR vs. Non-cardiac	Non-cardiac	P value Cardiac AL vs. Non-cardiac
Interventricular septal thickness in diastole (IVSd) cm (CI)	<0.001	1.52 (1.48, 1.56)	<0.001	1.67 (1.62, 1.72)	<0.001	1.09 (1.06, 1.12)	<0.001
Left ventricular internal dimension in diastole (LVIDd) cm (CI)	0.001	4.13 (4.03, 4.22)	0.005	4.38 (4.26, 4.50)	0.99	4.36 (4.25, 4.46)	0.004
Left ventricular posterior wall thickness in diastole (LVPWd) cm (CI)	<0.001	1.44 (1.39, 1.47)	<0.001	1.61 (1.56, 1.64)	<0.001	1.04 (1.01, 1.07)	<0.001
LV Ejection Fraction % (CI)	<0.001	52 (50, 54)	<0.001	45 (43, 48)	<0.001	60 (59, 61)	<0.001
Mitral valve deceleration time (MVdecT) ms (CI)	<0.001	189 (180, 199)	0.93	198 (186, 210)	<0.001	236 (224, 247)	<0.001
E/E' (CI)	<0.001	16.9 (15.7, 18.1)	0.99	16.9 (15.7, 18.1)	<0.001	8.82 (8.2, 9.4)	<0.001
E/A Ratio (IQR)	<0.001	1.48 (0.91, 2.36)	0.045	2.17 (1.03, 2.70)	<0.001	0.85 (0.73, 1.06)	<0.001
Iso-volumetric relaxation time (IVRT) ms (CI)	<0.001	85.3 (80.54, 90.16)	0.99	85.3 (79.93, 90.82)	0.001	98.8 (94.55, 103.05)	<0.001
Tissue Doppler imaging lateral wall (TDIs) m/s (CI)	<0.001	0.07 (0.06, 0.07)	0.26	0.05(0.04, 0.06)	<0.001	0.09 (0.09, 0.10)	<0.001
Small complexes on ECG, N (%)	<0.001	76 (50.7)	<0.001	22 (25.3)	0.99	42 (30.4)	<0.001

Table 5.3 Baseline Symptoms and Cardiac Medications in Patients who had Holter Monitoring

History	Overall P value	Cardiac AL	P value (cAL vs. cATTR)	Cardiac ATTR	P value (cATTR vs. Non cardiac)	Non-cardiac AL	P value (cAL vs. Non-cardiac)
Ischaemic Heart Disease, N (%)	0.162	19 (12.4)		16 (17.8)		13 (9.2)	
Previous Arrhythmia, N (%)	<0.001	35 (23)	<0.001	48 (53.3)	<0.001	19 (13.5)	0.15
Hypertension, N (%)	0.004	43 (28.1)	0.777	32 (36)	0.414	66 (46.8)	0.003
Palpitations, N (%)	0.328	20 (13.1)		10 (11.1)		11 (7.8)	
Shortness of Breath, N (%)	<0.001	116 (75.8)	0.99	70 (77.8)	<0.001	49 (34.8)	<0.001
Syncope, N (%)	0.498	11 (7.2)		8 (8.9)		7 (5.0)	
NYHA, N (%) 1	<0.001	25 (16.7)	0.99	15 (17.4)	<0.001	76 (55.9)	<0.001
2		71 (47.3)		52 (60.5)		46 (33.8)	
3		49 (32.7)		15 (17.4)		12 (8.8)	
4		3 (2.0)		4 (4.7)		1 (0.7)	
MAYO stage, N (%)	<0.001		0.99		<0.001		<0.001
1		49 (34.5)		38 (42.7)		124 (89.9)	
2		4 (2.8)		2 (2.2)		1 (0.7)	
3		89 (62.7)		49 (55.1)		13 (9.4)	
Total Blood Pressure agents, N (%) 0	<0.001	73 (48)	<0.001	20 (22.7)	<0.001	67 (47.5)	0.99
1		54 (35.5)		29 (33)		41 (29.1)	
2		22 (14.5)		31 (35.2)		25 (17.7)	
3		3 (2)		7 (35.2)		2 (1.4)	
4		0 (0)		0 (0)		4 (2.8)	
5		0 (0)		1 (1.1)		1 (0.7)	
6		0 (0)		0 (0)		1 (0.7)	
Beta Blocker, N (%)	<0.001	43 (28.7)	0.002	44 (49.4)	<0.001	26 (18.7)	0.195
Digoxin, N (%)	0.024	4 (2.7)	0.057	9 (10.1)	0.258	5 (3.6)	0.99
Amiodarone, N (%)	0.197	6 (4)		2 (2.2)		1 (0.7)	
Other anti-arrhythmic agents, N (%)	0.682	2 (1.3)		0 (0)		2 (1.4)	
Aspirin, N (%)	0.552	36 (24)		27 (30.3)		38 (27.3)	
Angiotensin II Receptor Blocker, N (%)	0.038	12 (8)	0.105	16 (18)	0.99	23 (16.5)	0.123
Calcium Channel Blocker, N (%)	0.001	6 (4)	0.174	10 (11.2)	0.702	25 (18)	<0.001
Other Blood Pressure Medications, N (%)	0.012	0.99 (0.7)	0.441	3 (3.4)	0.99	10 (7.3)	0.027
Loop diuretic, N (%)	<0.001	99 (66)	0.363	68 (76.4)	<0.001	59 (42.4)	<0.001
Thiazide diuretic, N (%)	0.575	7 (4.7)		4 (4.5)		10 (7.2)	
Other diuretic, N (%)	<0.001	30 (20.1)	0.99	21 (23.6)	<0.001	4 (2.9)	<0.001

Frequency of Arrhythmias

Ten percent of cATTR patients had a pacemaker at the initial assessment, significantly more than cAL (1.3%; P=0.009) and non-cardiac patients (0.7%; P=0.003) (Table 5.4). Supra-ventricular tachycardia was uncommon, occurring in only ~7% of all patients with no significant difference in prevalence between the three patient groups. Arial fibrillation was present in 42.7% of cATTR patients, significantly more than cAL (18.3%; P<0.001) which was in turn more frequent than non-cardiac patients (7.8%; P=0.03). Significantly more complex ventricular arrhythmias (ventricular grading 3-5) were seen on Holter monitoring in cATTR patients (64.8%) compared to cAL (44.8%; P=0.003) and non-cardiac (25%; P=0.003). Non sustained VT (grade 5) was seen in 29.7% of cATTR, 23.7% of cAL and 8.3% of non-cardiac patients. No patient had sustained VT defined as lasting for more than 30 seconds and no patient reported syncope on their diary sheet.

The mean IVSd was significantly higher among patients with complex ventricular arrhythmias compared to patients without complex ventricular arrhythmias (Mean IVSd 1.30cm (CI 1.26, 1.35) vs. 1.52cm (CI 1.46, 1.57)(P<0.001). There was no significant difference in mean IVSd between cAL patients with and without complex ventricular arrhythmias (Mean (CI) 1.57cm (1.49, 1.65) vs. 1.48cm (1.43, 1.53), P=0.06), and similarly no difference in wall thickness among cATTR patients with and without complex ventricular arrhythmias (Mean (CI) 1.68cm (1.61, 1.71) vs. 1.65cm (1.56, 1.53), P=0.54). There was no correlation between severity of cardiac involvement as assessed by the MAYO staging system and frequency of complex ventricular arrhythmias. It seems therefore, that the amyloid fibril type (more frequent in cATTR) is

potentially more important than disease severity in predicting risk of complex ventricular arrhythmias.

Table 5.4 Frequency of Arrhythmias on Holter Monitoring

	Overall P value	Cardiac AL	P value (cAL vs. cATTR)	Cardiac ATTR	P value (cATTR vs. non-cardiac)	Non-cardiac AL	P value (cAL vs. Non cardiac)
Pacemaker, N (%)	<0.001	2 (1.3)	0.009	9 (10)	0.003	1 (0.7)	1
Supraventricular Tachycardia, N (%)	0.967	12 (7.8)		7 (7.8)		10 (7.1)	
Bradycardia, N (%)	0.249	9 (5.7)		10 (11.1)		8 (5.5)	
Complex Ventricular arrhythmia, N (%)	<0.001	70 (44.8)	0.003	61 (64.8)	<0.001	36 (25)	0.003
Atrial Fibrillation, N (%)	<0.001	28 (18.3)	<0.001	38 (42.7)	<0.001	11 (7.8)	0.039
Ventricular Grading*, N (%)	<0.001		0.006		<0.001		0.003
0		32 (20.5)		10 (10.6)		48 (33.1)	
1		50 (32.7)		19 (21.1)		57 (40.2)	
2		4 (2.6)		3 (3.3)		3 (2.1)	
3		2 (1.3)		2 (2.2)		3 (2.1)	
4		31 (20.3)		31 (34.4)		21 (14.9)	
5		37 (23.7)		28 (29.7)		12 (8.3)	

* Grade 0: No ventricular premature depolarisations. Grade 1: Occasional ventricular premature depolarisations no more than 30 per hour. Grade 2: More than 30 ventricular polarisations per hour. Grade 3: Multiform ventricular depolarisations. Grade 4: Couplets (two consecutive ventricular depolarisations). Grade 5: Non sustained ventricular tachycardia (three or more consecutive ventricular beats at a rate of ≥120 beats/min, lasting for <30 seconds).

Patient Outcome

Median follow-up from Holter monitoring for the whole study population was 14 months (IQR 8.5, 21.8), during which there were 111 (28.9%) deaths; 73 (46.7%) cAL, 15 (15.9%) cATTR, and 23 (16.3%) non-cardiac. Median Kaplan Meier survival was not reached in any group (Figure 5.1); however, survival estimates were significantly worse in cAL than in both cATTR (Mean (CI) 18.1 months (15.9, 20.2) vs. 25 months (22.9, 27); Log rank P<0.001) and non-cardiac patients (Mean (CI) 26.8 (25.2, 28.4); Log rank P<0.001). There was no difference in estimated survival between cATTR and non-cardiac patients.

One hundred and thirty four patients were taking anti-arrhythmic agents at their initial assessment. There was no difference in overall survival between patients prescribed anti-arrhythmic agents and patients not taking anti-arrhythmics. One hundred and thirteen patients were taking B-blockers; there was no difference in survival between patients on and off B-blockers. None of a total of 9 patients taking amiodarone died during follow-up.

On univariate analysis of the whole cohort the only arrhythmia associated with significantly worse survival was atrial fibrillation (18.0 months (CI 15.3, 20.85) vs. 24.5 months (CI 23.2, 25.8); P<0.001). Figure 5.2 shows Kaplan Meier survival in the whole cohort stratified by grading of ventricular arrhythmias, there was a significant trend towards worse survival as grade of ventricular arrhythmia increased, but no individual grading was associated with significantly worse survival than another.

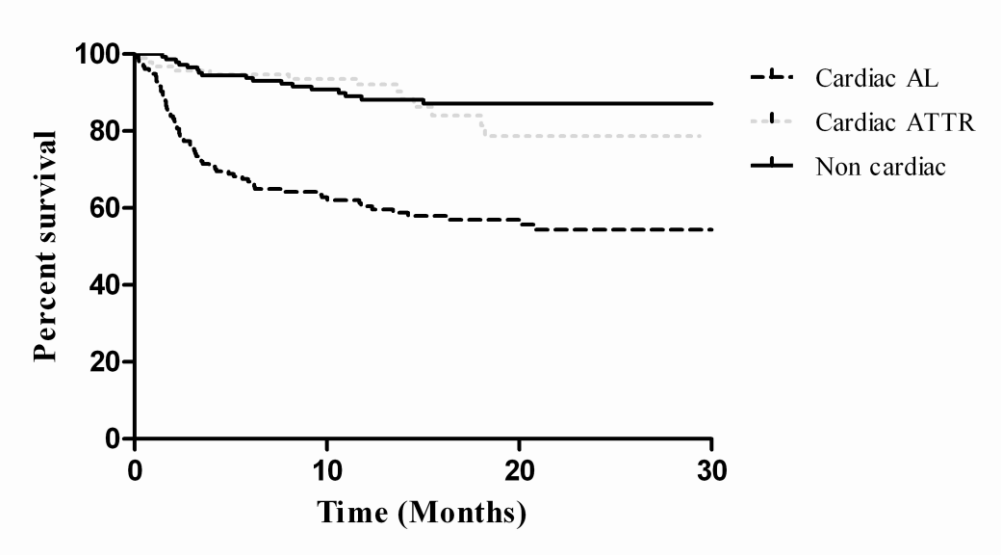
Atrial fibrillation remained associated with poorer survival in cAL (Mean (CI) 10.4 months (6.5, 14.4) vs. 20.1 months (17.7, 22.57) and non-cardiac patients (Mean (CI) 18.7 months (12.5, 23.9) vs. 27.4 months (25.8, 28.9); P =

0.015, but was not associated with a worse outcome in the cATTR patients (Mean (CI) 23.1 months (19.6, 26.6) vs. 26.6 months (25.8, 28.9). Among cAL patients, as the grading of ventricular arrhythmias increased, survival appeared to reduce (Table 5.5). Grading of ventricular arrhythmias did not correlate with survival in the cATTR patients; there were too few events in this cohort of patients to draw any definitive conclusions. There was no difference in survival when the whole cohort was stratified for the following parameters: presence or absence of a pacemaker, and presence or absence of SVT/complex ventricular arrhythmia. Similarly, these parameters did not appear to influence survival even when those taking anti-arrhythmic medications were excluded from the analysis.

Table 5.5 Survival Estimates in Patients with Cardiac AL Amyloidosis by Grading of Ventricular Arrhythmia

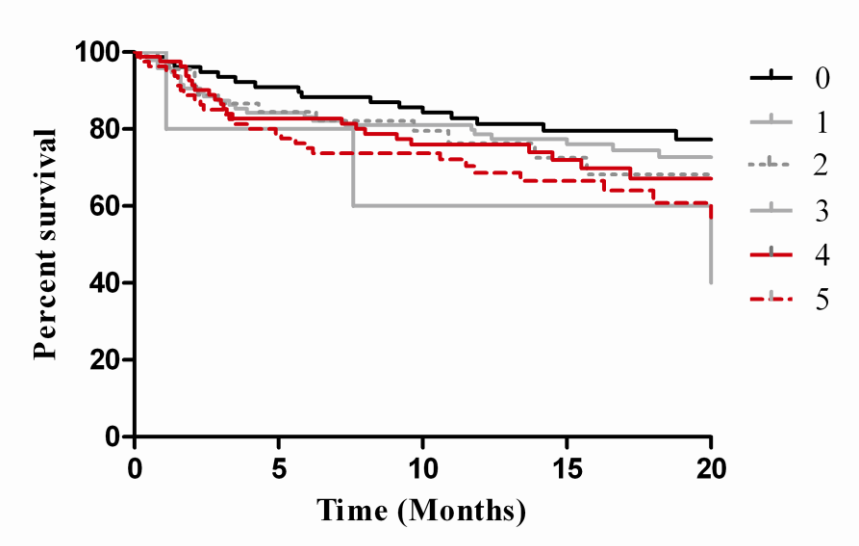
Ventricular Arrhythmia Grading in patients with Cardiac AL Amyloidosis	Mean Survival (months)	Lower bound 95% CI	Upper Bound 95% CI	P value (Log Rank)
0	21.8	17.1	26.4	0.049
1	18.7	14.2	23.1	
2	17.7	11.7	23.8	
3	15.3	0.01	35.1	
4	15.5	11.3	19.8	
5	15.0	10.7	19.4	

Figure 5.1 Patient Survival from Holter Monitoring Stratified by Cardiac Amyloid Type



Log Rank test for trend P <0.0001

Figure 5.2 Patient Survival in Total Cohort of Patients Stratified by Ventricular Grading



Log Rank Test for trend P = 0.01

Non Sustained Ventricular Tachycardia

A significant proportion of patients were discovered to have grade 5 ventricular arrhythmias. Table 5.6 outlines the proportion that had triplets, salvos or more than 5 beats of non-sustained ventricular tachycardia. In the cATTR group 14.8% of patients had more than 5 beats of non-sustained VT, compared to only 8.3% of cAL patients, and 0.7% of non-cardiac patients. In most cases only one run of >5 beats of VT was identified during the 24 hours of monitoring. Seven of 13 (53.8%) of cAL patients who had >5 beats of non-sustained VT died, compared to only 1 of 14 (7.1%) cATTR patients and 1 patient in the non-cardiac group. Only 1 patient reportedly had a cardiac arrest; he was successfully resuscitated on two occasions and subsequently died in a hospice. No other patient who had >5 beats of non-sustained VT on their Holter had a sudden cardiac death.

Eleven of 14 patients with cATTR amyloidosis who had >5 beats of VT were taking B-blockers at the time of their first 24 hour Holter monitor. Based on the Holter findings, therapeutic alterations were recommended to the treating physician; namely either to increase the dose of B-blocker or to change to amiodarone. Five such patients had their medications altered. Among cAL patients, 3 of those with >5 beats of VT were already taking B-blockers at the time of Holter monitoring and 5 commenced B-blockers after the finding of non-sustained VT. Three patients with cAL amyloidosis had repeat Holter monitoring during which no further episodes of non-sustained VT were identified. Five cATTR patients had repeat Holter monitoring; 4 of 5 showed further episodes of non-sustained VT.

Table 5.6 Type of Non Sustained Ventricular Tachycardia and Frequency of Events

Ventricular Tachycardia breakdown	Cardiac AL (N = 37)	Cardiac TTR (N = 28)	Non-Cardiac (N = 12)
Triplets only	10 (6.4)	6 (6.3)	5 (3.4)
Salvos only	4 (2.5)	4 (4.2)	1 (0.6)
Salvos and triplets	10 (6.4)	4 (4.2)	5 (3.4)
More than 5 beats of VT	13 (8.3)	14 (14.8)	1 (0.7)
Number of episode of more than 5 beats			
1	6 (3.8)	11 (11.7)	1 (0.7)
2	3 (1.9)	3 (3.1)	0 (0)
3	3 (1.9)	0 (0)	0 (0)
>3	1 (1.64)* 5 runs	0 (0)	0 (0)

*Triplets: three consecutive ventricular depolarisations, ** Salvos: four or five consecutive ventricular depolarisations.

Discussion

Complex ventricular arrhythmias are reportedly common in AL amyloidosis, occurring in 47% of cases in one study.[236] Studies to date have involved small patient numbers and have focussed predominantly on AL amyloidosis. Non-sustained VT was the most frequently identified arrhythmia among 24 patients who underwent autologous stem cell transplantation and were associated with a higher risk of death.[241] Data in patients with ATTR amyloidosis is extremely limited. Three of six patients in one study were shown to have ventricular tachycardia.[236] Patients with ATTR commonly have first and second degree heart block on the ECG. Prophylactic pacemakers were reported to prevent major cardiac events in 25% of 262 patients followed up in a recent study.[227] The results in chapter 4 showed that having a pacemaker was a poor prognostic feature in patients with ATTRwt possibly indicating that rhythm disturbances may be a potentially significant cause of death in this group. This study of 394 patients has

shown that arrhythmias are a common feature in patients with both cATTR and cAL, especially in cATTR. Complex ventricular arrhythmias were the most common finding and were present in 65% of cATTR patients and 45% of cAL patients followed by atrial fibrillation which featured in 43% of cATTR and 18% of cAL patients.

The cause of rhythm disturbances in amyloidosis is not well understood. In one study the hypothesis for the development of ventricular arrhythmias and sudden death was that microvascular amyloid deposition and inflammation possibly results in focal adrenergic receptor up-regulation and catecholamine hypersensitivity.[241] An alternative hypothesis is that infiltration of the His-Purkinje system may give rise to conduction abnormalities. Histologic examination has shown that amyloid fibrils infiltrate the cardiac conduction system and may preferentially involve His-purkinje fibres.[237, 238] Prolonged infra-His conduction times were commonly seen in one study of AL amyloidosis patients and were reported to be associated with sudden death.[242] One might therefore expect that patients with greater interstitial expansion and thicker myocardial walls may have greater infiltration of the His-purkinje system and that there may be an association with more frequent arrhythmias; however we could find no significant association between wall thickness and frequency of arrhythmias. Similarly the MAYO staging system is a very strong predictor of outcome in AL amyloidosis,[88] and a positive troponin T (effectively consistent with MAYO stage 3 disease) was a predictor of poor outcome in ATTRwt patients described in chapter 4. If rhythm disturbances are significantly associated with poor outcome one might expect that patients with a poorer prognosis by MAYO staging would have more frequent dysrhythmias. There was no

association in this study of more frequent complex ventricular arrhythmias and MAYO staging indicating that severity of cardiac disease does not necessarily correlate with arrhythmias.

The precise mode of death associated with cardiac amyloidosis is poorly understood. Electromechanical dissociation was reported by Kristen *et al* to be the cause of death in 7 of 19 patients who received prophylactic defibrillators. In the same study, two patients were successfully DC cardioverted following episodes of sustained ventricular tachyarrhythmias. This study concluded that prophylactic defibrillators are not beneficial for patients, however better selection of patients to identify those at risk of sustained ventricular tachyarrhythmias would aid in distinguishing patients who may benefit from insertion of prophylactic defibrillators.[228] Couplets, complex ventricular arrhythmias and ventricular tachycardia were all associated with worse survival in one study however, only couplets were predictive of sudden cardiac death in AL amyloidosis.[75] Median survival was not reached in any of the three groups in the study presented here and therefore robust conclusions cannot be made about predictors of survival. However atrial fibrillation and increasing grade of ventricular arrhythmia did appear to be associated with worse outcome in the cAL group.

Non sustained VT was more commonly seen in patients with cATTR. Runs of non-sustained VT were mostly isolated events and were not seen again on repeat Holter monitors. The subsequent management of patients was in line with current guidelines, most patients were started on a B-blocker or had the dose increased after non-sustained VT was identified, and this may have affected the frequency of events on repeat testing. Deaths in patients who had non-sustained

VT did not appear to be due to sudden cardiac events, however no deaths were monitored at the point of death and so the mode of death remains unknown.

Study Limitations

Median survival was not reached in this prospective study and therefore robust conclusions regarding predictors of patient outcome cannot yet be made. Patients were followed for a median of 14 months and analysis of results was performed 6 months after recruitment of the last patient. Among patients with cAL amyloidosis, we can be more confident of the findings since median survival in isolated cardiac AL amyloidosis patients was 10 months (see chapter 4) such that the follow up period was potentially adequate. Median survival in the ATTRwt patients is more than 2.5 years (see chapter 4), so the follow-up in this study is insufficient to draw any firm conclusions on risk factors for death.

Holter monitors were performed at the initial assessment and therefore it was not possible to stop anti-arrhythmic agents 48 hours prior to monitoring occurring. We do not know the degree to which agents such as B-blockers prevent arrhythmias and the baseline frequency of arrhythmias may well be greater when patients are not taking medications. This study was designed as a pilot study to decipher the frequency of rhythm disturbances in the types of amyloidosis which commonly affect the heart. It was deemed unethical not to treat patients in line with current guidelines when potentially harmful arrhythmias were identified. Recommendations were therefore made to treating physicians to commence anti-arrhythmic agents in patients who had non-sustained VT. The degree to which such therapies may have affected outcome remains unknown; a further study is therefore needed to ascertain whether anti arrhythmic agents are effective in cardiac amyloidosis.

This study was designed to measure the baseline frequency of arrhythmias in patients with cardiac amyloidosis. Patients were therefore recruited on the basis of suspected cardiac disease, and due to limited availability of holter monitors not all newly referred patients had holter monitoring on their first visit. This recruitment bias may influence the results as patients who were not suspected to have cardiac disease were excluded. It is possible that some patients with less severe cardiac disease may not have been included and patients who had Holters who were subsequently found not to have cardiac amyloid may be more likely to have previous cardiac arrhythmias. There were significantly more patients in the non-cardiac AL group with a history of hypertension. This is likely to reflect recruitment bias and is a confounding factor which may influence the frequency of arrhythmias seen in the non cardiac group.

A substantial proportion of patients had a previous history of ischaemic heart disease, a potentially significant confounding factor. The proportion of patients however with ischaemic heart disease was comparable in each group (P=0.162) it is possible that this could influence the overall frequency of arrhythmias, however it is unlikely to influence the differences between the three groups and therefore these patients were not excluded from analyses.

Results Section Three: Renal Amyloidosis

Chapter Six: Outcome in Renal AL Amyloidosis following Chemotherapy

Introduction

The first half of the thesis has concentrated on the epidemiology of amyloidosis and the cardiac disease phenotype. Comparison has been made between ATTRwt and cardiac AL amyloidosis. The focus of the thesis will now turn to the renal phenotype. ATTRwt is a predominantly cardiac disease and significant renal dysfunction is not seen. In AL amyloidosis renal involvement which frequently progresses to ESRF, is the commonest clinical manifestation of the disease. The diagnosis of AL amyloidosis is commonly obtained through renal histology.[243]

Over the past 20 years the use of cytotoxic chemotherapy to suppress production of amyloidogenic monoclonal immunoglobulin has improved outcomes[78, 131,244-246] such that median survival in AL amyloidosis now exceeds three years.[247,248] Features at presentation that predict renal response[249] and progression to dialysis have recently been reported,[127] but modifiable factors that may influence renal outcome have been little studied and findings in the small published series are conflicting.[103] Furthermore, the few studies of renal replacement therapy in AL amyloidosis have mostly comprised small numbers opatients with dismal survival.[103,104,250] The aims of chapter 6 are to identify factors at baseline presentation that are associated with patient survival and renal outcome, and to assess outcome on dialysis in patients with AL amyloidosis.

Methods

Patients

We included in this study all 923 patients who were diagnosed with renal AL amyloidosis and were assessed for the first time at the NAC between March 1987 and May 2008. Renal involvement in AL amyloidosis was defined as non-Bence Jones proteinuria of >0.5 g/day according to the amyloidosis international consensus criteria.[60] The diagnosis of AL amyloidosis was confirmed histologically in 853 cases and through a series of non-invasive investigations in the remaining 70 cases. In the absence of a histological diagnosis of AL amyloidosis, the following criteria were required for inclusion in the study: amyloid deposition in the bones and viscera on ^{123}I-labeled SAP scintigraphy; renal dysfunction fulfilling the amyloidosis international consensus criteria; evidence of a clonal B cell dyscrasia; absence of amyloidogenic mutations[44] and absence of a chronic inflammatory disorder, or acute phase response.

Patients underwent prospective protocolized 6 monthly assessments at the NAC.

Assessment of Hematologic Response

For all patients whose baseline visit to the NAC was after December 2001, serial FLC concentration was prospectively monitored on blood samples scheduled monthly during periods of chemotherapy treatment and 1-3 monthly during subsequent follow up. For patients who were first evaluated before this time, FLC concentration was determined retrospectively wherever possible using archived serum samples obtained at the baseline NAC assessment and first follow up visit 6 months later.

Healthy, polyclonal serum FLC concentrations rise progressively through advancing stages of CKD[206] which impedes the monitoring of monoclonal light chain disorders. In this study, the value of the FLC 'monoclonal component' was estimated by subtracting the concentration of the uninvolved light chain from that of the amyloidogenic light chain, the FLC difference (dFLC), a strategy that has been validated in myeloma.[207] The FLC response to chemotherapy was defined throughout the study as the percentage of the dFLC at baseline that remained at the time of analysis. In order to validate the method in AL amyloidosis, dFLC response was included as a covariate in the patient survival analysis.

FLC response was 'time updated' in analyses of patient and renal survival and was defined at 6 months from baseline in analyses of renal outcome by amyloidosis international consensus criteria.[60]

Assessment of Renal Outcome

Risk factors for progression to dialysis were analyzed among the 752 patients with a baseline eGFR of ≥15 ml/min, but not among the remaining 171 patients (Table 6.1).

A separate analysis of factors influencing change in renal function from baseline according to the amyloidosis international consensus criteria and including both 'renal response' and 'renal progression' was undertaken in all 429 patients with follow up renal data. Patients who died (n=226) or started dialysis (n=111) within 6 months of their baseline NAC visit and those in whom there was no creatinine or proteinuria measurement recorded after 6 months from baseline (n=157) were excluded from this analysis (Table 6.1). A 6 month time point from baseline was chosen because it corresponded to the first follow up visit during

which FLC response was routinely determined, and also allowed sufficient time for occurrence of a potential change in renal function. Renal progression was defined as the earliest of: (i) starting dialysis; (ii) 50% increase in proteinuria AND increase by ≥1 g/day; or (iii) 25% increase in serum creatinine AND follow up creatinine >120 μmol/L.[60] Renal response was defined as the earliest of: (i) 50% decrease in proteinuria AND decrease by ≥0.5 g/day as long as creatinine had not increased by 25%; or (ii) 25% reduction in serum creatinine as long as proteinuria had not increased by 50%.[60]

Survival Analyses and Statistical Methods

Patient follow up was censored at date of last clinic visit before May 2008. Kaplan-Meier analyses and Cox regression were used to investigate factors associated with overall survival of all patients in the cohort. Cox regression models were also used to: identify independent predictors of progression to dialysis-dependence among 752 patients with eGFR ≥15 ml/min at baseline, indentify predictors of either a 'renal response' or a 'renal progression' among 429 patients; and define survival from commencement of dialysis among 221 patients (Table 6.1). All factors which were of statistical and/or clinical significance (P<0.20) in univariate analyses were included in multivariate analyses. Cut points were chosen by their clinical relevance (ALP, bilirubin, albumin, CKD stage, and proteinuria) or on the basis of previously published work in amyloidosis (NT-proBNP, FLC concentration). The log rank test was used to compare difference in stratified Kaplan-Meier survival analyses.

Table 6.1 Summary of Cohort and Sub Group Analysis in Patients with Renal AL Amyloidosis

Analyses	Patients Included and Excluded from Analysis		Patient No.
Factors influencing patient survival	**Exclusion**	None	0
	Inclusion	All patients in the study	923
Factors influencing progression to dialysis	**Exclusion**	All patients with eGFR <15 ml/min at baseline	171
	Inclusion	All patients with eGFR ≥15 ml/min at baseline	752
Factors influencing change in renal function	**Exclusion**	Patients who died before 6 months follow up	226
		Patients who progressed to dialysis before 6 months follow up	111
		Patients without follow up renal data at or after 6 months	157
	Inclusion	All patients with follow up renal data at or after 6 months	429
Factors influencing patient survival from dialysis	**Exclusion**	Non-dialysis patients	702
	Inclusion	All dialysis patients	221

Results

Baseline Characteristics and Patient Survival

Baseline characteristics of the patients are listed in Table 6.2. The cohort of 923 patients were followed for a median (IQR) of 22.4 (7.3, 49.0) months after diagnosis. A total of 530 (57.4%) patients died. The Kaplan-Meier median survival from diagnosis for the whole cohort was 35.2 (95% CI: 28.0, 42.0) months (Figure 6.1). Median (IQR) time from diagnosis to baseline NAC visit was 2.8 (1.4, 5.8) months.

NT-proBNP concentration at baseline was strongly associated with survival (Figure 6.2) and NT-proBNP >150 pmol/L remained significantly associated with mortality in multivariate analyses (P<0.0001). There was a clear decreasing trend between risk of death and magnitude of FLC response to chemotherapy, with a hazard ratio for death of 0.73 (95% CI: 0.57, 0.92, P=0.01) for patients achieving a 50-90% FLC response, and 0.27 (95% CI: 0.18, 0.39, P<0.0001) for those achieving >90% FLC response compared to patients achieving <50% FLC response. Other baseline factors associated with increased mortality were older age, lower urine protein, bilirubin >21 μmol/L, ALP >130 u/L, systolic blood pressure <120 mmHg, lower serum albumin and higher amyloidogenic serum FLC concentration (Table 6.3).

Table 6.2 Baseline Characteristics of Patients with Renal AL Amyloidosis (N=923)

		N (%)
Sex	Male	524 (56.8)
Age (years)	Median (IQR)	62 (55, 70)
Time from diagnosis to baseline evaluation (months)	Median (IQR)	2.8 (1.4, 5.8)
Year of amyloid diagnosis	≤2000	312 (33.8)
	2001-2004	377 (40.9)
	2005-2008	234 (25.4)
Amyloid load by SAP scan	Small	434 (47.1)
	Moderate	222 (24.1)
	Large	216 (23.4)
	Missing	51 (5.5)
Pre-treatment with chemotherapy	Yes	329 (35.7)
Chronic kidney disease stage	1	141 (15.3)
	2	263 (28.5)
	3	256 (27.7)
	4	92 (10.0)
	5	146 (15.8)
	Missing	25 (2.7)
Started dialysis before first visit		94 (42.5)
Started dialysis within 6 months of first visit		42 (19.0)
Started dialysis after 6 months from first visit		85 (38.5)
Serum albumin (g/L)	<25	332 (36.0)
	26-35	354 (38.4)
	>35	221 (23.9)
	Missing	16 (1.7)
24 hour urine protein (g)	0.5-3	264 (28.6)
(BJP not included)	3.1-10	520 (56.3)
	>10	109 (11.8)
	Missing	30 (3.3)
NT-proBNP (pmol/L)	0-35	97 (10.5)
	36-150	95 (10.5)
	151-400	76 (8.2)
	>400	145 (15.7)
	Missing	510 (55.3)
Amyloidogenic light chain	Lambda (%) / Kappa (%)	77.3% / 22.7%
Laboratory markers at first visit		**Median (IQR)**
Haemoglobin (g/dL)		12.5 (11.1, 14.0)
Serum creatinine (µmol/L)		106 (78, 192)
Creatinine clearance (ml/min)		54.4 (28.5, 81.6)
eGFR (ml/min)		56 (27, 79)
24 hour urine protein (g)		5.1 (2.5, 7.6)
Serum albumin (g/L)		28 (23, 35)
Bilirubin (u/L)		7 (5, 11)
Alkaline phosphatase (u/L)		107 (76, 175)
Systolic blood pressure (mmHg)		125 (110, 145)
Diastolic blood pressure (mmHg)		76 (68, 85)
Standing systolic blood pressure (mmHg)		123 (109, 140)
Standing diastolic blood pressure (mmHg)		76 (66, 86)
Lambda light chain in lambda patients (mg/L)		99.1 (37.5, 283.5)
Kappa light chain in kappa patients (mg/L)		18.7 (9.7, 52.7)

Figure 6.1 Kaplan-Meier survival from diagnosis in systemic AL amyloidosis. Median (IQR) survival among 923 patients was 35.2 (28.0, 42.2) months.

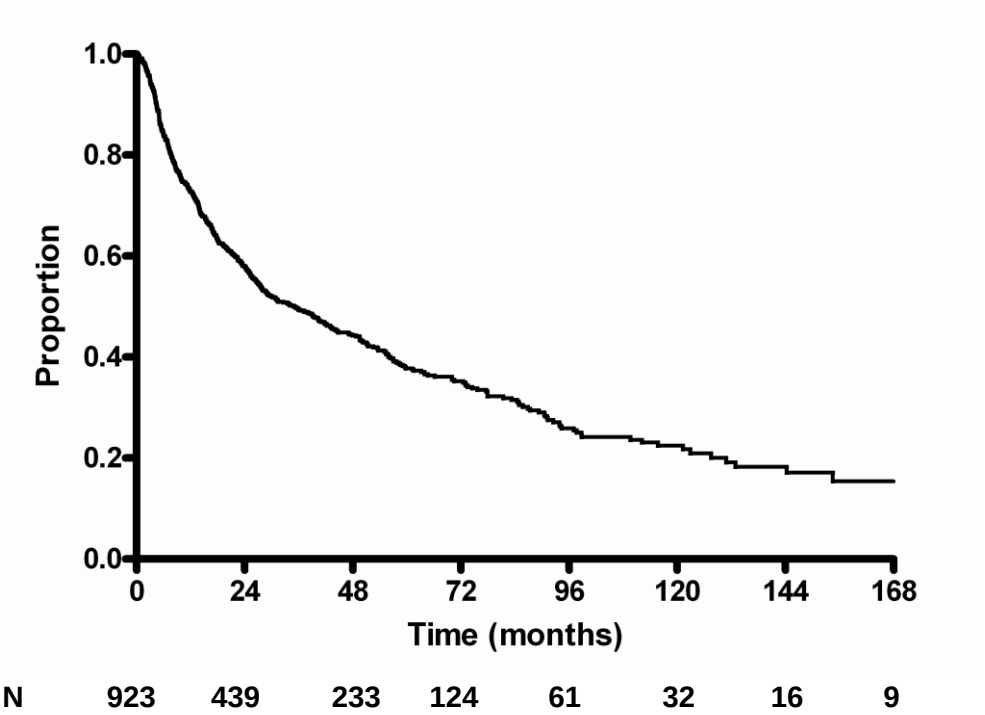


Figure 6.2 Survival from diagnosis stratified by NT-proBNP. Survival was significantly better among patients with baseline NT-proBNP <150 pmol/L (black line) compared to those with NT-proBNP >150 pmol/L (grey line) (median 97.0 vs 35.9 months; P<0.0001, log rank test).

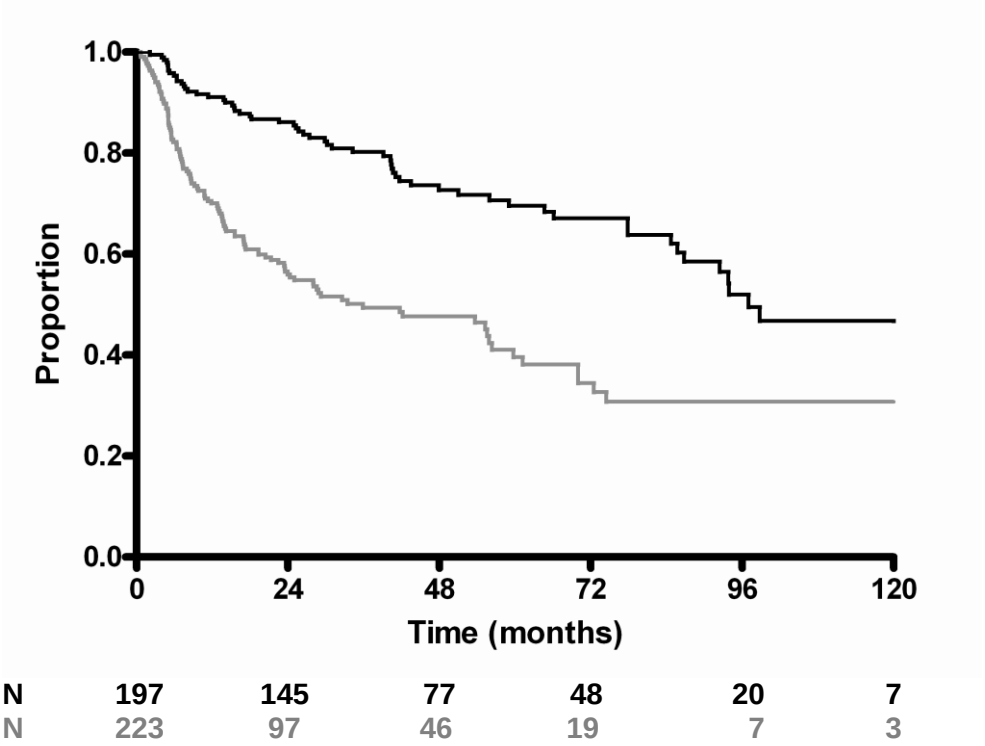


Table 6.3 Factors Associated with Death in 923 Patients with Renal AL Amyloidosis

			Univariate		Multivariate	
		Died/Total (%)	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
Dialysis (time-updated)	No	476/829 (57.4)[1]	1	0.02	1	0.06
	Yes	54/94 (57.4)	1.29 (1.04, 1.59)		0.77 (0.58, 1.01)	
Sex	Male	314/524 (59.9)	1.27 (1.07, 1.51)	0.01	1.20 (1.00, 1.43)	0.05
	Female	216/399 (54.1)	1		1	
FLC response (time updated)	<50%	N/A[2]	1	-	1	-
	50-90%		0.70 (0.55, 0.88)	0.05	0.73 (0.57, 0.92)	0.01
	>90%		0.31 (0.21, 0.44)	0.003	0.27 (0.18, 0.39)	<0.0001
	Missing		1.27 (1.00, 1.62)	<0.0001	0.82 (0.63, 1.08)	0.16
Yr of baseline evaluation	<2002	255/356 (71.6)	1.15 (0.96, 1.37)	0.12	0.60 (0.48, 0.76)	<0.0001
	≥2002	275/567 (48.5)	1		1	
Age at diagnosis	Per 10 yr Higher		1.22 (1.12, 1.33)	<0.0001	1.22 (1.11, 1.33)	<0.0001
NT-proBNP at baseline (pmol/L)	0-35	25/101 (24.8)	0.22 (0.14, 0.35)	<0.0001	0.33 (0.20, 0.53)	<0.0001
	36-150	34/96 (35.4)	0.39 (0.26, 0.58)		0.40 (0.26, 0.61)	
	151-400	32/78 (41.0)	0.49 (0.33, 0.74)		0.56 (0.37, 0.85)	
	>400	83/148 (56.1)	1		1	
	Missing[3]	356/500 (71.2)	1.22 (0.96, 1.56)		1.15 (0.88, 1.51)	
Baseline FLC concentration (mg/L)	0-150	116/291 (39.9)	1	<0.0001	1	<0.0001
	151-500	155/273 (56.8)	1.96 (1.54, 2.49)		1.79 (1.39, 2.30)	
	>500	123/190 (64.7)	2.27 (1.76, 2.93)		2.30 (1.76, 3.01)	
	Missing	136/169 (80.5)	3.99 (3.10, 5.12)		3.04 (2.27, 4.07)	
CKD Stage at baseline	1-3	375/660 (56.8)	1	0.005	1	0.002
	4-5	140/242 (57.9)	1.26 (1.04, 1.53)		0.87 (0.68, 1.12)	
	Missing	15/21 (71.4)	1.96 (1.17, 3.28)		2.47 (1.42, 4.30)	
24 hr urine protein at baseline (g/24 hr)	0-3	166/264 (62.9)	1.37 (1.13, 1.65)	<0.0001	1.34 (1.08, 1.66)	<0.0001
	3-10	289/520 (55.6)	1		1	
	>10	56/109 (51.4)	0.78 (0.59, 1.04)		0.60 (0.44, 0.82)	
	Missing	19/30 (63.3)	1.94 (1.22, 3.08)		1.76 (1.03, 3.00)	
Bilirubin at baseline (u/L)	0-21	472/836 (56.5)	1	<0.0001	1	0.004
	>21	46/65 (70.8)	2.16 (1.59, 2.92)		1.52 (1.08, 2.13)	
	Missing	12/22 (54.5)	1.12 (0.63, 1.98)		4.09 (1.26, 13.24)	
Albumin at baseline (g/L)	<20	90/129 (68.8)	1.49 (1.16, 1.90)	0.01	2.73 (2.04, 3.63)	<0.0001
	20-29	212/363 (58.4)	1.22 (1.01, 1.48)		1.56 (1.27, 1.92)	
	>30	221/419 (52.7)	1		1	
	Missing	7/12 (58.3)	1.08 (0.51, 2.30)		0.62 (0.14, 2.75)	
ALP at baseline (u/L)	0-130	307/581 (52.8)	1	<0.0001	1	<0.0001
	>130	214/326 (65.6)	1.81 (1.52, 2.16)		1.76 (1.45, 2.14)	
	Missing	9/16 (56.3)	1.03 (0.53, 2.01)		0.33 (0.06, 1.89)	
Systolic BP at baseline (mmHg)	<120	140/243 (57.6)	1	<0.0001	1	<0.0001
	>120	132/323 (40.9)	0.63 (0.49, 0.79)		0.72 (0.56, 0.93)	
	Missing	258/357 (72.3)	1.55 (1.26, 1.91)		1.48 (1.18, 1.86)	

[1]Numbers refer to dialysis at any time during follow up. [2]Not applicable since 'time-updated' and patients may move from one FLC response category to another. [3]Despite missing NT-proBNP data in 500 patients, there was no statistical difference in survival between 'missing' and <35 pmol/L categories.

Renal Outcome: Factors Influencing Progression to Dialysis

Among 752 patients with an eGFR of ≥15 ml/min at baseline, 98 (13.0%) progressed to ESRF and received dialysis after a median of 26.8 months. Independent factors at baseline associated with progression to dialysis were higher CKD stage (HR for CKD stage 3: 2.06 (1.22, 3.49), and CKD stage 4: 7.07 (4.01, 12.47) compared to CKD stage 2; P<0.0001) and lower serum albumin (3.04 (1.57, 5.88) for albumin <20 g/L compared with >30 g/L; P=0.003). FLC response was also significantly associated with progression to dialysis; patients with a 50-90% response (0.63 (0.39, 1.00), P=0.05) and those with a >90% response (0.24 (0.12, 0.49), P=0.0001) were less likely to progress to dialysis compared to patients with <50% response.

Renal Outcome: Factors Influencing Change in Renal Function

Among 429 patients evaluable for renal outcome by amyloidosis international consensus criteria, progression of renal disease from baseline occurred in 235 (54.8%) patients and renal responses occurred in 140 (32.6%) cases. Interestingly, CKD stage at baseline did not significantly influence the chance of renal response with 27.3%, 33.5%, 33.6%, and 27.8% of patients with CKD stages 1, 2, 3 and 4 respectively achieving a renal response. The median time from baseline NAC visit to renal progression was 23.8 (95% CI: 18.8, 33.5) months. In univariate analyses, FLC response at 6 months from baseline (HR=0.32 (95% CI: 0.20, 0.52) for >90% response compared to <50% response), 24hr urine protein (1.62 (1.15, 2.27) for urine >10 g compared with <3 g) and serum albumin (2.47 (1.71, 3.58) for albumin <20 g/L compared with >30 g/L)

were associated with an increased risk of renal progression. Factors significantly associated with renal response and renal progression in multivariate analyses are shown in Table 6.4. Importantly, achieving >90% FLC response at 6 months was associated with a near four-fold increase in the chance of renal response (P<0.0001) and a 68% reduction in the chance of renal progression (P<0.0001) when compared to an FLC response of 0-50%. In sensitivity analyses, renal outcome was significantly better among patients with a >90% FLC response compared to those with 50-90% FLC response (P=0.0004 for renal progression; P<0.0001 for renal response).

Among 253 non pre-treated patients, HR for renal progression among 50-90% FLC responders was 0.67 (0.44, 1.02) and for >90% FLC responders was 0.26 (0.14, 0.50) compared to <50% FLC responders (P=0.0002). HR for renal response among non pre-treated patients who were 50-90% FLC responders was 1.56 (0.77, 3.13) and for >90% FLC responders was 4.29 (2.15, 8.57) compared to <50% FLC responders (P<0.0001).

Table 6.4 Independent Factors Significantly Associated with Renal Progression and Renal Response among 429 Evaluable Patients

		Renal Progression			Renal Response		
		N (%)	**HR (95% CI)**	**P-value**	**N (%)**	**HR (95% CI)**	**P-value**
FLC response at 6 months	0-49%	77/120 (64.2)	1	**<0.0001**	23/120 (19.2)	1	**<0.0001**
	50-90%	97/163 (59.5)	0.76 (0.56, 1.04)		48/163 (29.4)	1.64 (0.99, 2.71)	
	>90%	21/65 (32.3)	0.32 (0.19, 0.52)		41/46 (89.1)	3.95 (2.36, 6.63)	
	Missing	40/81 (49.4)	0.52 (0.35, 0.78)		28/81 (34.6)	1.76 (0.98, 3.16)	
CKD stage	1	39/88 (44.3)	0.68 (0.47, 1.01)	**0.003**	Excluded from multivariate analyses[1]		
	2	88/164 (53.7)	1				
	3	71/119 (59.7)	1.24 (0.90, 1.71)				
	4	23/37 (62.2)	1.76 (1.09, 2.84)				
	5	9/14 (64.3)	1.71 (0.84, 3.45)				
	Missing	5/7 (71.4)	2.27 (0.91, 5.69)				
Serum albumin	<20 g/L	42/54 (77.8)	2.74 (1.76, 4.27)	**<0.0001**	9/54 (16.7)	0.33 (0.16, 0.69)	**0.01**
	20-29 g/L	101/169 (59.8)	1.90 (1.37, 2.63)		43/169 (25.4)	0.57 (0.38, 0.85)	
	≥30 g/L	88/202 (43.6)	1		86/202 (42.6)	1	
	Missing	4/4 (100)	2.11 (0.72, 6.16)		2/4 (50.0)	0.58 (0.08, 3.99)	

235/429 (54.8%) experienced renal progression and 140/429 (32.6%) experienced renal response [1]P=0.67 in univariate analyses

Dialysis Outcome

Two hundred and twenty-one of 923 (23.9%) patients with renal AL amyloidosis received dialysis during the course of their disease, 127 only after their first visit to the NAC. One hundred and fourteen of 221 (51.6%) dialysis dependent patients died and median survival from commencement of dialysis, which was unaltered by censoring at renal transplantation, was 39.0 (95% CI: 29.8, 43.9) months (Figure 6.3). Survival stratified by year of commencement of dialysis is shown in Figure 6.4. Serum albumin <25 g/L (P=0.04) and alkaline phosphatase >130 u/L at commencement of dialysis (P=0.02) were significantly associated with mortality in multivariate analyses.

Figure 6.3 Kaplan-Meier survival from commencement of dialysis in systemic AL amyloidosis. Median survival among all (n=221) patients from start of dialysis was 39.0 months.

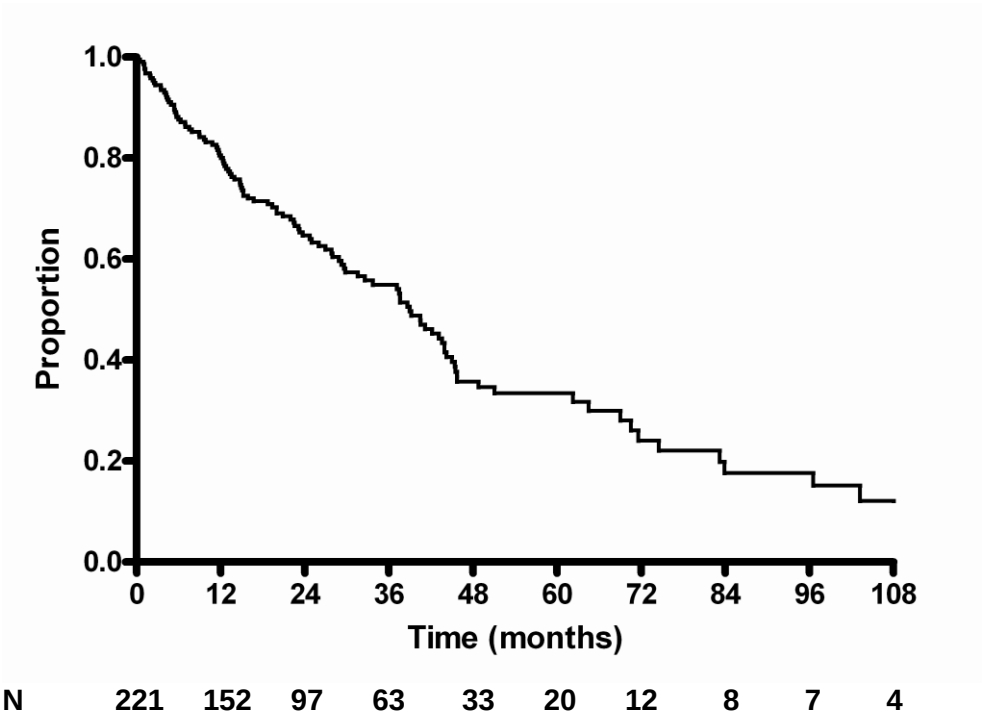
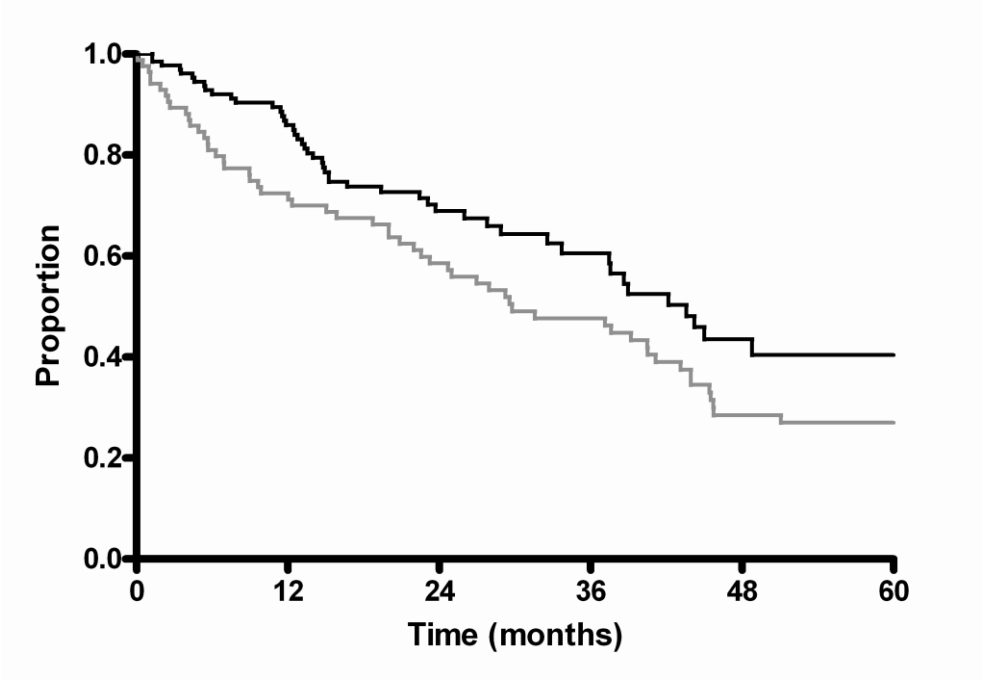


Figure 6.4 Patients who started dialysis after 2002 (black line) survived for significantly longer than those starting dialysis before 2002 (grey line) (median 43.6 vs 29.8 months; P=0.05).



Discussion

This study, comprising long-term follow up of a uniquely large cohort of 923 patients with renal AL amyloidosis, demonstrates prolonged survival and superior renal outcomes among patients who achieved greater than 90% reduction in their dFLC value following chemotherapy. The substantial survival advantage associated with an FLC response demonstrated here is completely consistent with previous studies in AL amyloidosis,[78, 125] and the present series therefore validates the use of the dFLC method in this disease. As CKD progresses, the concentration of healthy polyclonal and abnormal monoclonal FLCs increase progressively, reflecting reduced renal clearance and metabolism, invalidating the use of absolute FLC measurements for tracking hematologic response in patients with renal disease. In contrast, the validity of dFLC measurements throughout all stages of CKD enabled estimation of the proportion of the amyloidogenic monoclonal light chain present at baseline that remained after chemotherapy. This has demonstrated for the first time a compelling and substantial relationship between magnitude of FLC response to chemotherapy and renal outcome. FLC response at 6 months from baseline was independently associated with both improvement in renal function and renal progression among evaluable patients in this series with a >90% response conferring a near fourfold chance of renal response and a greater than 3 fold reduction in chance of renal progression compared to <50% FLC response ($P<0.0001$). Other factors independently associated with renal progression and/or requirement for dialysis included lower eGFR, lower serum albumin and heavier proteinuria at presentation, consistent with previous reports.[127] It is however important to recognize that management and outcome of individual patients is also governed by tolerability and toxicity of

treatment, and that the design of the present study did not ascertain renal progression or deaths that may have resulted from counterproductive attempts to translate a clonal PR to CR with additional chemotherapy. It is also important to appreciate that the degree to which monoclonal light chain concentration needs to be suppressed in order to facilitate improvement in amyloidotic organ dysfunction differs not only between individuals but also between different organs within the same individual. The present findings therefore do not imply that chemotherapy should necessarily target a >90% FLC response in all patients with renal AL amyloidosis, but, subject to prospective validation, the present findings support this general objective in the absence of undue toxicity from chemotherapy, guided by serial FLC measurements using the simple dFLC method. The low proportion of patients reaching dialysis in this cohort in comparison to other series is likely to reflect the markedly different inclusion criteria; one cannot directly compare patients with renal AL amyloidosis according to amyloidosis international consensus criteria with patients who present with dominant renal AL amyloidosis.

Independent factors associated with death in this series, along with <50% FLC response to chemotherapy, were older age, higher absolute baseline amyloidogenic FLC concentration and lower serum albumin at presentation. In addition, systolic blood pressure <120 mmHg, higher NT-proBNP, and alkaline phosphatase and bilirubin outside the normal range at presentation, which are likely to reflect amyloidotic involvement of the autonomic nerves, heart and liver respectively, were associated with poorer survival.

Median survival from commencement of dialysis among 221 patients was >3 years, considerably longer than previously reported,[103,104,127,250] and was 43.6 months for patients starting after 2002. The reasons for the prolonged

survival on dialysis in comparison to other series as well as the improved survival on dialysis among those commencing after 2002 remain unclear but, in the absence of evidence that the criteria for instituting dialysis differ or have changed over time respectively, are likely to reflect a combination of improved supportive management, improved dialysis techniques and better chemotherapy treatments for AL amyloidosis.

Median survival from dialysis was not changed when censoring for transplantation. This may suggest that transplantation does not confer a survival benefit over dialysis, or too few patients may have been transplanted for a modest survival benefit to have an impact on the results. The results following transplantation will be described further in the next chapter.

Chapter Seven: Renal Transplantation in Systemic Amyloidosis – Importance of Amyloid Fibril Type and Precursor Protein Abundance

Introduction

The results of chapter 6 highlight the importance of suppression of the amyloid fibril precursor protein on patient outcome in AL amyloidosis. Progression to ESRF is common, in more recent years patients were shown to be surviving longer on dialysis. This has led to an increasing number of patients who may benefit from renal transplantation.

There are 7 types of systemic amyloidosis which commonly affect the kidneys and cause progressive renal dysfunction and ESRF. Disease natural history and organ involvement vary widely according to amyloid type. AA amyloidosis confers a better overall prognosis but is also typically associated with progression to ESRF which can be rapid.[86] AFib, which presents universally with proteinuria, is also characterized by a progressive decline in kidney function to ESRF within 5 years of diagnosis.[45] AApoAI is phenotypically heterogeneous but CKD is common and typically progresses more slowly to ESRF than AL, AA and AFib.[177] Likewise, ALys usually causes slowly progressive CKD.[180, 252]

The results of chapter 6 highlight the importance of suppression of the fibril precursor protein in AL amyloidosis with respect to altering disease natural

history and improving renal outcome. Similarly, suppression of SAA levels with anti-inflammatory therapy affects disease outcome in AA amyloidosis.[86] Fibrinogen A ∝-chain is synthesized by the liver alone[253] and approximately 50% of plasma apolipoprotein A-I is liver derived.[176] Orthotopic Liver Transplantation (OLT) therefore results in complete replacement of variant amyloidogenic fibrinogen in AFib and partial replacement of variant apoAI in AApoAI by the respective normal protein, and thus halts or slows amyloid accumulation.[165, 177, 254] It is not currently possible to alter the natural history of ALys.

Renal transplantation remains a contentious issue in patients with systemic amyloidosis. This is predominantly due to fears of early allograft loss from recurrent amyloid and poor outcomes related to progressive extra-renal amyloidosis. Concerns that immunosuppression could increase the malignant potential of low grade plasma cell dyscrasias have also been expressed although remain largely unfounded. Patient survival in AL amyloidosis has improved in association with an ever increasing armamentarium of available chemotherapeutic agents[130] and similarly, there are new therapeutic options for patients with chronic inflammatory diseases.[255] These advances mean that there are more ESRF patients with stable extra-renal AL and AA amyloid deposits. A recent study of renal transplantation in AA amyloidosis found that 14% of patients developed recurrent disease in their graft which was associated with an increased risk of death. It was therefore hypothesized that there may be an association between SAA concentration and recurrent disease, this has yet to be systematically tested.[105]

The aim of this chapter is to characterize outcome following renal transplantation in the different forms of renal amyloidosis and ascertain whether

suppression of the precursor protein prior to transplantation confers a benefit to graft outcome.

Materials and Methods

Patients

All patients who received either a renal transplant (RTx) or combined liver kidney transplantation (CLKT) between January 1978 and May 2011 were identified from the NAC database. The amyloid type was confirmed in each patient by review of histology[200] and where appropriate, by additional genetic analysis, as previously described.[208] The diagnosis of amyloidosis had been established prior to renal transplantation in 91 patients and only after renal transplantation in 13 cases.

Patients with AL amyloidosis were reviewed at the NAC every 6 months and those with AA and hereditary amyloidosis were reviewed annually.

Assessment of Fibril Precursor Protein Abundance

The clonal response was defined according to the minimum response achieved after renal transplantation (e.g. a complete clonal response must have been achieved throughout follow-up) by previously published criteria[60] adapted for use in CKD[256] as follows: CR was a normal FLC ratio and no detectable serum paraprotein or BJP, no response (NR) was a dFLC concentration of >50% of the pre-treatment value, and partial response (PR) was a dFLC concentration of <50% the pre-treatment value in the absence of CR.

Among patients with AA amyloidosis response was defined according to serial SAA concentration, measured at each NAC appointment and at monthly intervals in between.[86] Median SAA concentration was calculated for each year after renal transplantation and response was defined according to the minimum response after renal transplantation as follows: CR was a median SAA <10 mg/L, PR was defined as median SAA from 10-50 mg/L and NR >50 mg/L, as previously described.[85]

Fibrinogen is made exclusively by the liver and the amyloidogenic variant protein disappears from the plasma after liver transplantation.[253] Liver transplantation in AApoAI results in a 50% reduction in the plasma concentration of the amyloidogenic apoAI variant.[176]

Serum lysozyme concentration changes with GFR and is associated with allograft function following renal transplantation.[257] It is not known what proportion of variant lysozyme remains in the plasma after solid organ transplantation. Patients with ALys were therefore excluded from the analysis of outcome by precursor protein response.

Assessment of Recurrent Disease

Recurrent amyloid in the graft was defined by abnormal uptake of tracer in the transplant on SAP scintigraphy (23 cases) and was corroborated by histology showing amyloid in 7 such cases. Patients underwent renal biopsies where clinically indicated. All cases that had recurrent amyloid on biopsy were associated with abnormal uptake of tracer within the allograft on SAP scintigraphy.

Assessment of Organ Function

Renal allograft function was assessed at each NAC appointment by measurement of serum creatinine, MDRD eGFR, measured creatinine clearance and 24 hour urine protein. Graft failure was defined as the date of recommencement of renal replacement therapy (RRT).

Statistical Analysis

Results are expressed as median and IQR or percentage. Patients were censored at their last NAC clinic visit and patient and graft survival were estimated by Kaplan-Meier analyses. Graft survival was non-censored for death; whereby death with a functioning graft was classified as graft loss. The log rank test was used to compare the difference in stratified Kaplan-Meier survival analyses. Statistical analysis was with the Man Whitney U test as all data analyzed was non-parametric (Graph pad prism version 5, Graph Pad, San Diego, Calif., USA).

Results

One hundred and four patients with systemic amyloidosis received a total of 111 renal transplants including 10 CLKTs and one combined cardiac and renal transplant. Sixty patients (58%) were male and 29 (28%) patients received grafts from live donors. A small proportion (12%) of patients received pre-emptive renal transplants; most commonly those with ALys where the rate of renal decline is typically slow (Table 7.1). A variety of immunosuppression regimens were used in accordance with local protocols (Table 7.1). Transplant survival differed between the different amyloid types and there appeared to be an association between natural history of disease and transplant survival, such that the amyloidosis syndromes associated with gradual loss of native eGFR, ALys and AApoAI, were associated with longer transplant survival than those known to cause rapid eGFR loss, AL, AA and AFib (Figure 7.1, log rank P=0.03). Amyloid did not recur in the renal allograft of any patient who achieved complete suppression of the precursor protein throughout the duration of their follow up despite prolonged follow up after transplantation in certain patients (Figure 7.2).

Figure 7.1 Renal transplant survival in years, non-censored for death stratified by disease natural history. Median survival in apolipoprotein A-I and lysozyme amyloidosis (slow natural history) was significantly longer than AL, AA and fibrinogen amyloidosis (fast natural history) (Median survival 13.1 years vs. 8.3 years; P = 0.03).

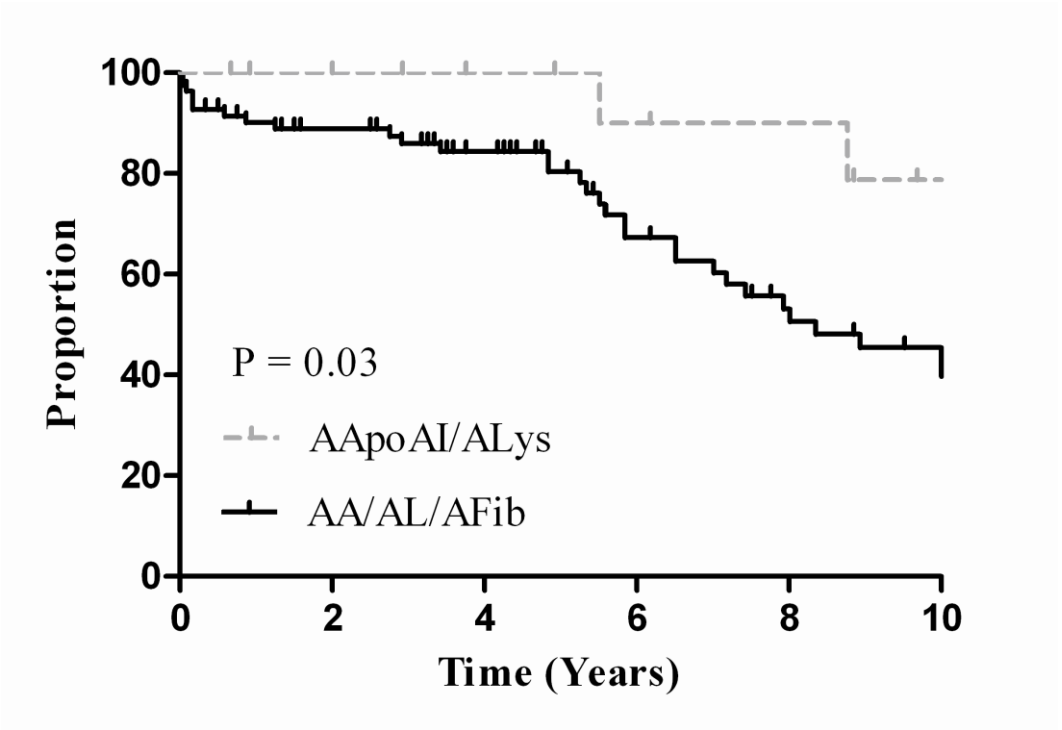
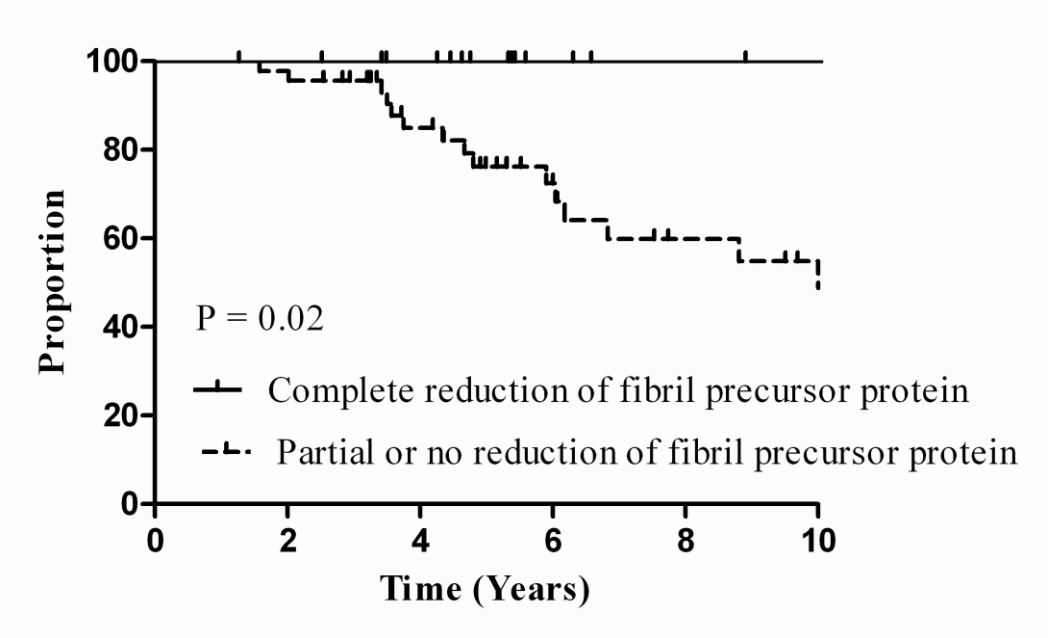


Figure 7.2 Time to recurrent amyloid in all patients by precursor protein abundance.



*Patients with combined liver kidney transplant in Fibrinogen amyloid are defined as CR and those with renal transplant NR and patients with Apolipoprotein AI amyloidosis are defined as PR

Table 7.1 Baseline Characteristics and Outcome of Patients who Underwent Renal Transplantation (N=104).

		No. of AL Patients N (%)	No. of AA Patients N (%)	No. of AFib Patients N (%)	No. of AApo AI Patients N (%)	No. of ALys Patients N (%)
Total Number of Patients		25	43	19	14	3
Total number of grafts		25	46	21	16	3
Total Number of Combined liver kidney transplants		0	0	9	2	0
Sex	Male	9 (36)	27 (62)	12 (63)	10 (71)	2 (66)
Age a transplant (years)	Median (IQR)	60 (52-63)	37 (29-48)	59 (56-61)	49 (37-56)	45 (32-62)
Donors	Live	5 (20)	16 (35)	2 (9.5)	3 (14)	3 (100)
Pre-emptive	Yes	1 (4)	3 (6.5)	5 (24)	1 (6.25)	2 (66)
Time from diagnosis to ESRF (years)	Median (IQR)	1.1 (0.0-3.6)	1.4 (0-5.8)	1.06 (0.03-1.6)	0.99 (0-27.4)	10.6 (0-14.6)
Time from ESRF to first transplant (years)	Median (IQR)	2.3 (1.1-5.0)	1.5 (0.9-3.0)	1.3 (0-2.8)	2.5(0.5-3.2)	0 (0-0.2)
Amyloid load at presentation	Small	7 (28)	7 (16)	12 (63)	2 (14.5)	0 (0)
	Moderate	8 (32)	26 (60)	6 (31.5)	4 (28.5)	1 (33)
	Large	7 (28)	10 (23)	1 (5.5))	8 (57)	2 (67)
	Missing[2]	3 (12)	0 (0)	0 (0)	0 (0)	0 (0)
Other organ involvement at presentation	Cardiac	5 (20)	0 (0)	0 (0)	1 (7.1)	0 (0)
	Liver	12 (48)	11 (25.5)	1 (5.2)	12 (85.7)	3 (100)
	Spleen	21 (87.5)	43 (100)	17 (89.4)	14 (100)	2 (66.6)
	Adrenals	1 (4)	7 (16.2)	1 (5.2)	0 (0)	0 (0)
[3]Underlying disease	Hereditary fever syndrome		10 (23.2)			
	Inflammatory bowel disease		9 (20.9)			
	Inflammatory arthritis		16 37.2)			
	Recurrent infection		5 (11.6)			
	Castleman's		2 (4.65)			
	Unknown		1 (2.32)			
[4]Number of lines of treatment	0	3 (12)				
	1	12 (48)				
	2	6 (24)				
	3	2 (8)				
	4	2 (8)				
Immunosuppression regimen	CNI/Pred/MMF	7 (28)	9 (20.9)	5 (26.3)	6 (42.8)	2 (66.6)
	CNI/Pred	5 (20)	9 (20.9)	3 (15.7)	3 (21.4)	0 (0)
	CNI/MMF	2 (8)	2 (4.6)	1 (5.2)	1 (7.1)	1 (33.3)
	Aza/Pred	1 (4)	7 (16.2)	2 (10.5)	1 (7.1)	0 (0)
	MMF/Pred	0 (0)	1 (2.3)	0 (0)	0 (0)	0 (0)
	CNI only	2 (8)	3 (6.9)	0 (0)	0 (0)	0 (0)
	Sirolimus/Pred/MMF	2 (8)	3 (6.9)	1 (5.2)	0 (0)	0 (0)
	Sirolimus only	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)
	Pred only	0 (0)	0 (0)	0 (0)	1 (7.1)	0 (0)
	Unknown	5 (20)	9 (20.9)	7 (36.8)	2 (14.2)	0 (0)
Grafts with recurrent amyloid	Number	7 (28)	9 (19.5)	7 (33.3)	3(18.75)	0 (0)
	Missing	2 (8)	4 (8.6)	0 (0)	2 (12.5)	0 (0)
Follow-up post transplant (years)	Median (IQR)	4.6 (2.6-6.9)	5.1 (3.4-11.6)	3.3 (0.5-6.5)	9.8 (3-13.7)	2.9 (0.9-6.8)
Number of deaths		13 (52)	16 (37.2)	7 (36.8)	1 (7.1)	0 (0)

AL Amyloidosis

Twenty five of 246 (10.2%) patients with AL amyloidosis who received RRT underwent renal transplantation, among whom median time to ESRF from diagnosis of amyloidosis was 1.1 (IQR 0.0-3.6) years, and median time to transplantation from ESRF was 2.3 (IQR 1.1-5.0) years. Median follow-up from transplantation was 4.6 (IQR 2.6-6.9) years. Thirteen (52%) patients died, most commonly from infection (6 patients), with 1 patient each dying from GI blood loss and cardiac decompensation. The remainder (5 patients) died from unknown causes. No patient who died from infection was receiving chemotherapy at the time. At the time of transplantation, amyloid was present in the spleen, liver and autonomic nerves in 21, 12 and 2 patients respectively. Five patients had cardiac amyloidosis deemed 'mild' on the basis of their echocardiogram. ECOG performance status was <2 in every case. Presence of extra-renal involvement by amyloid did not significantly influence patient survival.

Median graft survival was 5.8 years, 5 and 10 year graft survival was 74% and 25% respectively. Two patients lost their grafts, one to chronic allograft nephropathy (CAN) after 2.9 years and one from recurrent transplant pyelonephritis and obstructive nephropathy after 0.9 years. No grafts failed from recurrent amyloid, despite presence in 7 (28%) patients of renal allograft amyloid, diagnosed a median of 5.9 (IQR 3.8-6.3) years after transplantation.

Twenty two patients (88%) received chemotherapy in total. Clonal response at the time of transplantation was variable; 5 (20%) patients were in a clonal CR, 13 (52%) were in a PR and 3 (12%) cases had not achieved any clonal response or had not been treated prior to renal transplantation (Table 8.2). Among 21 patients who were evaluable for clonal response, 2 died within one year of

RTx, 18 were followed for more than one year post-RTx, and one was lost to follow-up (Table 7.3). There was no significant difference in renal allograft survival between patients who were in CR at the time of transplantation and those in PR although patients who had not achieved at least a PR prior to transplantation had significantly worse graft survival (5.3 vs 8.9 years; P = 0.02) (Figure 7.3). Five patients (28%) had a clonal relapse following transplantation after a median of 2.0 years for which 4 patients received further chemotherapy, 3 achieving a subsequent CR and 1 a PR. No patient developed symptomatic myeloma during follow-up.

Table 7.2 Fibril Precursor Protein Response Prior to Renal Transplantation

Type of amyloid (precursor protein)		**Abundance of precursor protein**	**No. (%)**
*AL (serum free light chain)	Clonal response at transplantation	CR PR NR unknown	5 (20) 13 (52) 3 (12) 4 (16)
AA (Serum amyloid A protein)	Median SAA (mg/L) during 6 months prior to transplantation	<10 10-50 >50 unknown	13 (30.2) 14 (32.5) 4 (9.3) 12 (27.9)
AFib (variant fibrinogen)	CLKT Kidney only	Complete removal No reduction	9 (47.3) 10 (52.6)
AApoAI (variant ApoAI)	CLKT Kidney only	50% reduction No reduction	2 (12.5) 14 (87.5)
ALys (variant lysozyme)	Kidney only	No reduction	3 (100%)

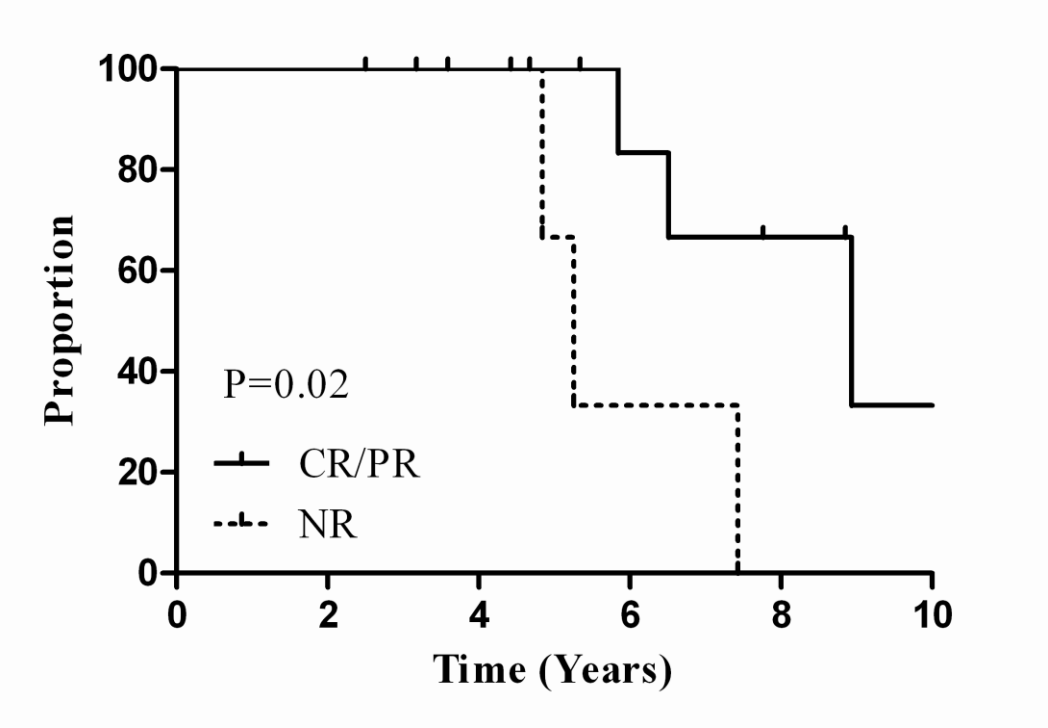
*In patients with AL amyloidosis; complete response (CR) is defined as normal free light chain ratio, no paraprotein or Bence Jones proteins, partial response (PR) is defined as dFLC response of >50% and no response (NR) a dFLC of <50%. (dFLC: the difference between the involved and uninvolved light chain).

Table 7.3 Response to Chemotherapy in 18 Evaluable Patients with AL Amyloidosis

Patient no.	Response at Tx	Number of years followed-up after transplant	Dead/Alive	Graft loss/ eGFR at last visit	Relapse Y/N (year after Tx patient relapsed	Chemotherapy after relapse	Clonal response after further treatment	Last known clonal response	Recurrent amyloid in graft at last visit (yes/no)
1	VGPR	6	Dead	33	No			VGPR	Yes
2	CR	8	Dead	18	No			CR	No
3	VGPR	8	Alive	49	Yes (2)	Intermediate dose Melphalan 4 cycles	CR	CR	No
4	VGPR	12	Alive	37	Yes (3)	CVAD 3 cycles	CR	CR	Yes
5	CR	6	Alive	39	No			CR	No
6	VGPR	2	Alive	55	Yes (1)	Bortezomib/ Dexamethasone 3 cycles	CR	CR	No
7	NR	5	Dead	30	No (*7)	Melphalan/ Dexamethasone 8 cycles	PR	PR	Yes
8	CR	4	Alive	41	No			CR	No
9	VGPR	4	Dead	78	No			VGPR	Yes
10	PR	3	Dead	48	Yes (1)	nil		NR	No
11	NR	3	Dead	42	No			VGPR	No
12	VGPR	1	Dead	58	Yes (2)	Cyclophosphamide /Dexamethasone 5 cycles	VGPR	VGPR	No
13	NR	4	Alive	45	No			VGPR	No
14	VGPR	4	Alive	48	No			VGPR	No
15	PR	8	Alive	33	No			VGPR	No
16	VGPR	5	Dead	37	No			VGPR	No
17	CR	4	Alive	45	No			CR	No
18	CR	5	Alive	26	No			CR	No

*Patient 7 wasn't treated with chemotherapy until progressive disease and recurrence in the graft was established 7 years after transplantation. CVAD - cyclophosphamide, vincristine, doxorubicin and dexamethasone, CR - complete response, VGPR - very good partial response, PR – partial response, NR – no response

Figure 7.3 Graft survival, non-censored for death in patients with AL amyloidosis according to clonal response at the time of transplantation.



AA Amyloidosis

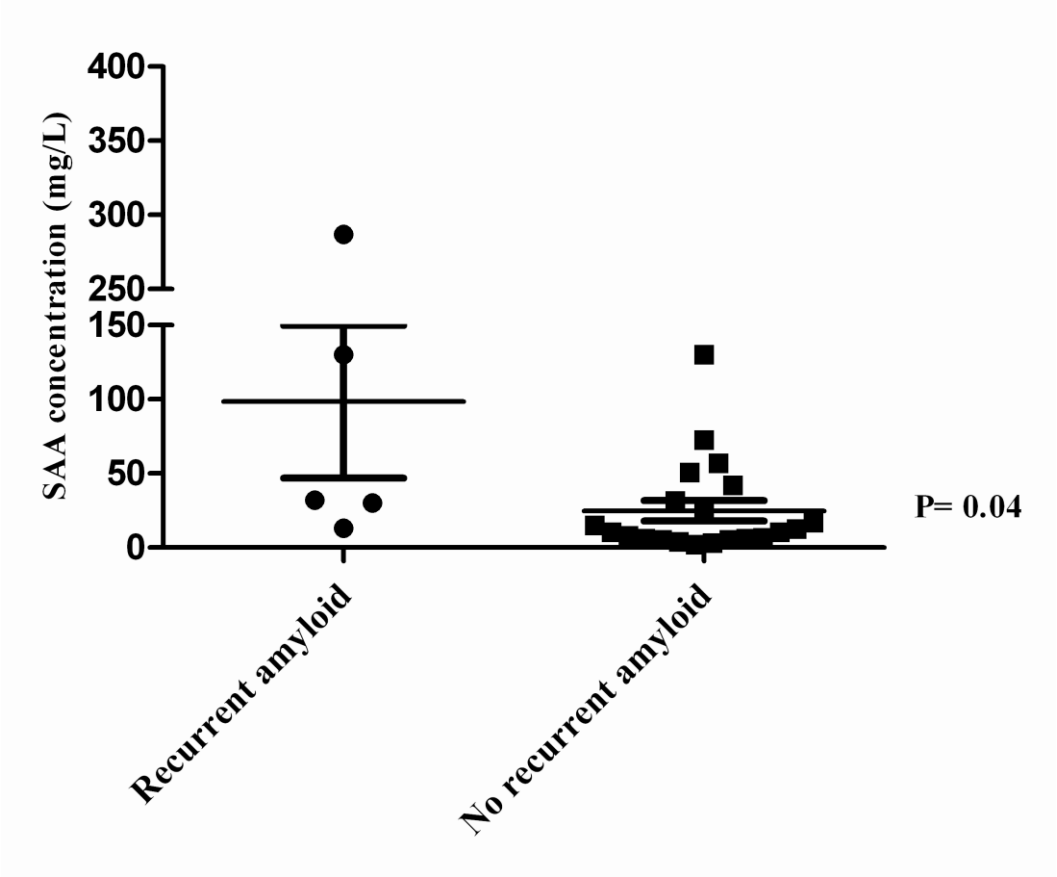
Forty three of 128 (33.6%) patients with AA amyloidosis who required RRT underwent renal transplantation, receiving a total of 46 grafts. The inflammatory diseases underlying AA amyloidosis included inflammatory arthritis in 16 (37.2%) patients, hereditary periodic fever syndromes in 10 (23.2%), inflammatory bowel disease in 9 (20.9%), recurrent infection in 5 (11.6%), Castleman's disease in 2 (4.7%) and was undefined in one patient. Median time from diagnosis to ESRF was 1.4 (IQR 0-5.8) years, and median time from ESRF to transplantation was 1.5 (IQR 0.9-3) years. Median follow-up from transplantation was 5.1 (IQR 3.4-11.6) years. Sixteen (37.2%) patients died, most commonly from infection (6 cases). One case each died from post transplant

lymphoproliferative disorder (PTLD), cervical malignancy, infarction of the transplant and bowel perforation and 6 from unknown causes.

Median estimated graft survival non-censored for death was 10.3 years and 8 (18.6%) grafts had failed at censor. Five and 10 year graft survival was 86% and 59% respectively. There was no significant difference in renal allograft survival between patients stratified by amyloid load or involvement of the liver, the latter being a measure of advanced AA amyloidosis.[258] Nine patients (19.5%) had recurrent amyloid in their renal allograft diagnosed a median of 5.3 (IQR 2.0-7.5) years after transplantation; 2 such cases lost their grafts due to recurrent amyloid. Three patients received a second RTx after failure of the first: one from primary non function whose second renal allograft lasted 17 years; one after 24 years from CAN whose second transplant is functioning well after one year; and one from recurrent amyloid after 8 years (six years after amyloid was first identified in the renal allograft) who died from sepsis one month after the second RTx.

Twenty-nine patients had serial SAA monitoring in the 6 months prior to transplantation. Graft survival non-censored for death was 14.5 years in patients with a median SAA value of <10 mg/L, and 7.8 years in those with a median SAA of >10 mg/L (P=ns). Median SAA concentration was significantly higher among those with recurrent amyloid in their graft compared to those in whom amyloid did not recur (P=0.04) (Figure 7.4).

Figure 7.4 Median serum amyloid A protein concentration measured during the 6 months prior to diagnosis of recurrent amyloid compared to the last 6 months of follow-up in patients without recurrent amyloid.



Hereditary Fibrinogen A∝-Chain Amyloidosis (AFib)

Nineteen of 51 (37.2%) patients with AFib who required RRT underwent renal transplantation receiving a total of 21 renal allografts including 9 patients who received CLKT. One patient received CLKT following failure of an initial isolated renal transplant after 5.6 years; CLKT was the initial transplant procedure among the remainder. Median time to ESRF from clinical presentation was 3.16 (IQR 1.5-7.8) years and median time from ESRF to transplantation was 1.4 (IQR 0-2.8) years. Four of 10 patients with RTx died, two from malignancy, one

following a GI bleed and one from an unknown cause. Among 9 patients who received CLKT, 3 (33.3%) died in the early post-operative course from peri-operative complications.

Median graft survival among patients who received isolated RTx (defined as NR) was 7.3 years compared to 6.4 years in those who received CLKT (defined as CR) (P=ns). Five and 10 year graft survival was 85% and 30% in patients with an isolated RTx, and 63% and 31% in those with CLKT. Recurrent amyloid was identified in the renal allografts of 7 patients, all of whom had isolated RTx, after a median of 4.9 (IQR 4.3-6.0) years. No patient with CLKT developed renal allograft amyloid. Four isolated RTx failed, 3 from recurrent amyloid and another from primary non function. One patient was discovered to have renal allograft amyloid at the time of graft failure, and graft loss occurred 1.2 and 2.5 years after discovery of amyloid in the remaining 2 cases. One CLKT patient lost their renal allograft after 6.5 years from CAN; none developed recurrent amyloid.

Hereditary Apolipoprotein A-I Amyloidosis (AApoAI)

Fourteen of 16 (87.5%) patients with AApoAI who required RRT underwent renal transplantation receiving a total of 16 renal allografts including 2 patients who received CLKT, one who was planned for CLKT but received the liver transplant alone due to intra-operative donor complications followed by a RTx 3 years later, and one who received a combined heart and renal transplant. Median time from clinical presentation to ESRF was 5.9 (IQR 4.2-10.8) years. Median time to transplantation from ESRF was 2.5 (IQR 0.5-3.2) years. One patient died during follow-up from an unknown cause 13 years after renal transplantation.

Median graft survival non-censored for death was 13.1 years, significantly longer than patients with AA, AL, and AFib amyloidosis (P=0.02). Five and 10 year graft survival was 100% and 77% respectively. Three patients (18.8%) all of whom had received isolated RTx, were found to have recurrent amyloid in their grafts after a median of 3.5 (IQR 2.8-17) years but this only caused graft loss in 1 case after a further 2 years of follow up with the other 2 cases losing their grafts after 8.8 and 12.5 years from chronic allograft nephropathy.

Hereditary Lysozyme Amyloidosis (ALys)

Three of 3 patients with ALys who required RRT received renal transplants, all from living donors. Two renal transplants were pre-emptive and the third patient only received dialysis for 2 months prior to RTx. Decline in native renal function was very slow; the median time from diagnosis to ESRF was 10.6 (IQR 0-14.6) years. All three grafts were functioning well 0.9, 2.9 and 6.2 years after RTx without evidence in any case of amyloid recurrence.

Discussion

This study details outcome associated with RTx in different types of renal amyloidosis and in particular, highlights the importance of the natural history of particular types of amyloidosis disease on outcome. The slowly progressive nature of ALys[252] and AApoAI[177] is well established and corroborated by a mean rate of eGFR loss among those with nephropathy visiting the NAC of only 4.7 ml/min/yr (95% CI 0.9-8.6 ml/min/yr). Despite the presence of extensive liver amyloid in most patients with these types of hereditary amyloidosis at the time of renal transplantation, and the persistent and undiminished production of the amyloid fibril precursor protein after the RTx procedure in all but 2 CLKT recipients, median estimated graft survival was 13.1 years with only 1/19 grafts lost to recurrent amyloid. There was only one death after median follow-up from transplantation of 8.8 (IQR 1.9-12.5) years. Furthermore, nearly all (17 of 19) patients with these types of amyloidosis who reached ESRF actually underwent renal transplantation.

These findings contrast AL, AA and AFib which are known to be associated with a more rapid disease natural history reflected by greater rates of eGFR loss.[45, 256] Until relatively recently there was a reluctance amongst nephrologists to proceed with renal transplantation in AA and AL amyloidosis due to the well documented risk of allograft failure from recurrent amyloid combined with the risk of death from progressive extra-renal amyloidosis.[259-261] Furthermore, amyloid typically recurs within renal transplants in AFib leading to graft failure between 5 and 12 years after RTx.[45] The natural history of AA, AL and AFib are modifiable however. Suppression of the precursor protein (dFLC) was shown in chapter 6 to be associated with improved patient and renal outcome.

Similarly in AA amyloidosis the SAA level is associated with renal outcome.[86] It stands to reason therefore, that the concentration of the fibril precursor protein is also likely to influence outcomes following renal transplantation. In AFib the precursor protein is made in the liver and liver transplantation therefore removes the amyloidogenic protein thus preventing ongoing amyloid accumulation.[262, 263] This study demonstrates good transplant outcomes among carefully selected patients with AA and AL amyloidosis such that only about one third of ESRF patients with AA amyloidosis and ~10% of ESRF patients with AL amyloidosis underwent renal transplantation. Median graft survival was 10.3 and 5.8 years in AA and AL patients in this series respectively. Amyloid was detected in the renal allografts of 9/43 (21%) and 7/25 (28%) patients with AA and AL amyloidosis respectively, but only 2 of these 68 grafts failed from recurrent amyloid despite up to 25 years of follow up. Graft outcomes were better among patients with suppression of the fibril precursor protein concentration prior to renal transplantation, and risk of amyloid recurrence in AA amyloidosis was higher among patients with elevated SAA levels. To what extent the fibril precursor protein needs to be suppressed in any particular individual in order to prevent recurrence in the renal allograft is not known. In this series there was no difference in renal allograft survival between AL amyloidosis patients who had achieved a clonal CR or clonal PR, although outcomes were poorer among those who had not achieved any clonal response prior to renal transplantation. Five (28%) patients experienced a clonal relapse following renal transplantation, and all 4 of those who received chemotherapy achieved at least a partial clonal response following further treatment, with no detrimental effect on renal allograft function. Based on this data, the recommendations would be to try and achieve at

least a partial clonal response in AL amyloidosis prior to listing for renal transplantation but that the absence of a complete clonal response should not preclude listing, particularly since it is usually possible to achieve a deeper clonal response with further chemotherapy once a patient has received a RTx. Current practice is to list patients immediately once a clonal response has been obtained.

The different amyloid types are associated with different patterns of organ involvement.[264] Furthermore, there is considerable heterogeneity in organ involvement, even within the same amyloid type. In this series, no relationship could be identified between patient survival and pattern of extra-renal organ involvement, nor total body amyloid burden. Presence of extra-renal amyloid should not therefore exclude patients from being listed for RTx. On the basis of this study the recommendation would be that as long as ECOG performance status and exercise tolerance are preserved, (thereby excluding patients with substantial cardiac and autonomic nerve involvement by amyloid), RTx should be considered, particularly if the extra-renal disease is stable in the context of a clonal response. It is notable however, that very few patients had cardiac amyloidosis which is known to confer a poor prognosis in AL amyloidosis[265] and is a rare complication of advanced AA amyloidosis.[266] Acute cardiovascular events were recently reported to be a significant cause of death among renal transplant recipients with AA amyloidosis.[105] There is little doubt that all patients with systemic amyloidosis should undergo an extensive cardiovascular assessment including specialized tests for presence and degree of cardiac amyloidosis prior to renal transplantation.

Chapter 8: General Conclusions

The studies described in this thesis outline several novel findings concerning the incidence, prognosis and management of systemic amyloidosis.

The epidemiology of amyloidosis in the UK has not previously been described, and there is historically only one publication from the US. Death certificates were shown here to be a reasonably sensitive method for detecting deaths attributable to systemic amyloidosis in England. Patients are being diagnosed with amyloidosis throughout England but there is a referral bias with significantly less patients from the North East, North West and Yorkshire seen at the NAC. The proportion of individuals diagnosed with systemic amyloidosis who are seen at the NAC is increasing year on year. The annual incidence of systemic amyloidosis in 2008 was estimated to be 10 per million population which is comparable to the historical estimate of disease incidence from the US.

ATTRwt is increasingly diagnosed in elderly patients attending cardiology clinics and the number of patients with the disease seen at the NAC is increasing dramatically. Accurately diagnosing fibril type in patients with isolated cardiac amyloidosis is paramount. Cardiac AL amyloidosis and ATTRwt are separate diseases with very different outcomes and management. Survival in ATTRwt is far superior to that in cardiac AL amyloidosis and chemotherapy is inappropriate in ATTRwt. Troponin T, the need for a pacemaker and severe functional impairment (NYHA class IV symptoms) are associated with poor outcome in ATTRwt. Age at diagnosis and level of NT-proBNP can help to distinguish between patients with ATTRwt and isolated cardiac AL amyloidosis when there is

an underlying clonal disorder. In patients with isolated cardiac amyloidosis and no clonal disease the chance of the amyloid type being AL is less than 1%.

Arrhythmic activity was frequently detected by 24 hour Holter monitoring among patients with cardiac amyloidosis. The most common abnormalities are complex ventricular arrhythmias and AF. There is a higher frequency of arrhythmic activity in cardiac ATTR amyloidosis than cardiac AL amyloidosis. Given that survival in AL amyloidosis is much shorter this may indicate that arrhythmic activity may not necessarily precipitate death in amyloidosis. The results of this Holter monitoring study could not determine a relationship between wall thickness or MAYO staging and frequency of complex ventricular arrhythmias, indicating that these two factors may not be a helpful way of identifying those who would benefit most from ICDs. Atrial fibrillation did appear to be associated with worse outcome; however median survival in this study was not reached. A further analysis after prolonged follow-up is necessary to determine outcomes and risk factors for death in cardiac amyloidosis.

The dFLC method is an appropriate way of evaluating haematologic response in patients with varying degrees of renal dysfunction in AL amyloidosis. Patients who achieve a >90% dFLC response have prolonged survival and better renal outcomes. The results presented in this thesis have changed current management of patients with AL amyloidosis in the UK. The dFLC method has routinely been incorporated into the response assessment. The target of chemotherapy remains a CR however this is often not achievable without precipitating major toxicity in patients with AL amyloidosis, and a 90% dFLC response is now deemed sufficient in the vast majority of cases to allow treatment to be discontinued. Similarly during relapse further chemotherapy is now

frequently instituted when the light chain levels have risen to a <90% dFLC response (in those who achieved this initially) rather than waiting for a doubling of the serum free light chain as was historically recommended.

Survival on dialysis has improved in recent years. Overall survival outcomes on dialysis among patients with AL amyloidosis were vastly superior to previous reports. This is likely to reflect a combination of more appropriate patient selection and improvements in supportive care, dialysis technique and advances in treatment with chemotherapy.

Progression of extra-renal amyloid and risk of recurrent disease in the renal allograft have, until recently, been major barriers to renal transplantation in systemic amyloidosis. No patient whose precursor protein was completely suppressed, regardless of amyloid type, developed recurrent disease in their graft. In diseases such as AL and AA amyloidosis where the precursor protein concentration can be modified by therapeutic intervention, it is important to monitor precursor protein concentration before and after renal transplantation. In AFib the only currently available treatment to suppress precursor protein concentration is liver transplantation, and the high risk of peri-operative mortality with CLKT must be balanced against elimination of risk of recurrent amyloid disease in the allograft further down the line; current recommended practice is to consider CLKT in younger, AFib patients.[45] In the hereditary amyloidoses with a slow natural history such as ALys and AApoAI, there is no need to suppress the precursor protein supply in order to prevent amyloid recurrence in the renal allograft since, in keeping with the disease natural history, this occurs extremely slowly.[177,252] When considering any patient with systemic amyloidosis for renal transplantation, a search for presence of cardiac amyloidosis is mandatory.

Future Studies

The results of chapter 4 described the presenting features and survival in ATTRwt amyloidosis. This is the largest retrospective study to date, and whilst it provides an initial insight into the disease there is much to learn about the natural history and long term follow-up. A prospective study of patients diagnosed with ATTRwt and hereditary TTR amyloidosis is planned. The aims of this study are to characterize the phenotype and clinical course of the disease in detail. Patients will be reviewed every six months and undergo serial CMR and ^{99m}Tc-DPD scintigraphy, as well as quality of life assessments.

The NAC has seen a massive increase in the number of patients with ATTRwt referred from cardiology clinics in the last 2 years. The true prevalence of the disease in the elderly population remains to be determined. ^{99m}Tc-DPD scintigraphy has proven to be a useful tool for diagnosing transthyretin amyloidosis, and uptake into the myocardium appears to be very sensitive. Patients who present currently with cardiac amyloidosis, routinely have both SAP and ^{99m}Tc-DPD scintigraphy as part of their initial assessment at the NAC. A further study is planned to estimate the prevalence of the disease in the elderly (over 80s). Subjects will be recruited from two groups; firstly ^{99m}Tc-DPD scintigraphy will be performed in 150 subjects from the general population with no prior diagnosis of IHD or CCF, and secondly a further 150 will be recruited from open access echocardiography and cardiology clinics at the Royal Free Hospital. Patients who have positive ^{99m}Tc-DPD scintigraphy will then be offered definitive investigations to diagnose the amyloid type and offered follow-up.

The model in chapter 4 utilizes age and NT-proBNP to give a probability of either ATTRwt or AL amyloidosis. ^{99m}Tc-DPD scintigraphy was not performed

in the majority of the cohort as it was not available. Now that ^{99m}Tc-DPD scintigraphy is a routine test it would be interesting to develop a model which utilizes all three measures in order to increase the sensitivity and specificity of the model, and to prospectively assess the predictive power of these basic tools, in order to reduce the need for biopsies.

In chapter 5 it was determined that arrhythmias are a common occurrence in cardiac amyloidosis, especially in ATTR type. The length of follow-up was insufficient to determine whether this has a significant impact on patient outcome, and more specifically sudden cardiac death. Further analysis is planned after prolonged follow-up to determine whether complex ventricular arrhythmias are associated with worse outcome.

Little is known about the mode of death in patients with cardiac amyloidosis. Implantation of REVEAL devices into patients with a poor prognosis i.e. MAYO stage 3 in AL patients, and patients with a positive Troponin T and NYHA class IV in ATTRwt could potentially identify the cardiac rhythm at the time of death. These devices could also determine whether malignant arrhythmias predate sudden cardiac death, and therefore identify patients who might benefit from an ICD.

The results of chapter 6 validated the dFLC method in AL amyloidosis and showed that a 90% reduction in dFLC conferred a greater chance of renal response. Since this data was published this target has been used in clinical practice. A prospective AL chemotherapy (Alchemy) study at the NAC has subsequently recruited 500 patients with systemic AL amyloidosis; this longitudinal observational study will determine the amount of treatment required and the toxicity associated with achieving this clonal response. It will also identify

the clonal responses that are actually achieved given this target and the rates of organ responses.

Publications Arising From This Thesis

Senile Systemic Amyloidosis: Clinical Features at Presentation and Outcome
Jennifer H Pinney, MRCP, Carol J Whelan, MD, MRCP, Aviva Petrie, Jason Dungu, MRCP, Sanjay M Banypersad, MRCP, Prayman Sattianayagam, MD, MRCP, Ashutosh Wechalekar, MD, MRCP, FRCPath, Simon DJ Gibbs, FRACP, FRCPA, Christopher P Venner, MD, Nancy Wassef, Carolyn A McCarthy, Janet A Gilbertson, Dorota Rowczenio, Philip N Hawkins, PhD, FMedsci, Julian D Gillmore, MD, PhD, FRCP , Helen J Lachmann, MD, FRCP. Journal of the American Heart Association. 2013 Apr 22;2(2) (Original Article)

Systemic Amyloidosis in England: An Epidemiological Study.
J.H. Pinney, C.J. Smith, J.B. Taube, H.J. Lachmann, C.P Venner, S.D.J. Gibbs, J Dungu, SM Banyperasad, A.D. Wechalekar, C.J. Whelan, P.N. Hawkins and J.D. Gillmore. British Journal of Haematology. 2013 May;161(4):525-32. (Original Article)

Systemic AA Amyloidosis.
Pinney JH, Lachmann HJ. Subcell Biochem. 2012;65:541-64. (Book Chapter)

Renal Transplantation in Systemic Amyloidosis-Importance of Amyloid Fibril Type and Precursor Protein Abundance.
Pinney JH, Lachmann HJ, Sattianayagam PT, Gibbs SD, Wechalekar AD, Venner CP, Whelan CJ, Gilbertson JA, Rowczenio D, Hawkins PN, Gillmore JD. American Journal of Transplanationt. 2013 Feb;13(2):433-41 (Original Article)

Outcome in renal AL amyloidosis after chemotherapy.
Pinney JH, Lachmann HJ, Bansi L, Wechalekar AD, Gilbertson JA, Rowczenio D, Sattianayagam PT, Gibbs SD, Orlandi E, Wassef NL, Bradwell AR, Hawkins PN, Gillmore JD. Journal of Clinical Oncology. 2011 Feb 20;29(6):674-81. (Original Article)

Amyloidosis.
Pinney JH, Hawkins PN. Annals of Clinical Biochemistry. 2012 May;49(Pt 3):229-41. (Review Article)

Amyloidosis and the lung.
Pinney JH, Lachmann HJ. European Respiratory Society Monograph 2011; 152-170; doi:10.1183/1025448x.10008010 (Monograph)

Paraprotein-related renal disease and amyloid.
Jennifer H. Pinney, Helen J. Lachmann Medicine 2011 August, 39 (8), 481-485 (Review Article)

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Appendix 1

Patient details/sticker
Name _____
DOB _____
Hospital number _____

Holter Monitoring Patient Sheet

What is Holter monitoring?
This device monitors your heart beat. It provides information about the rate and rhythm of your heart when you are active and records each beat your heart makes over the next 24 hours. By keeping a diary whilst the monitor is recording your heart beat we can assess if any changes to your heart beat occur and when this happens.

What do I need to do?
The monitor will be removed when you come back to clinic tomorrow. Please do not remove it yourself. Please keep the monitor dry at all times, if one of the leads falls off please replace it in the same position as soon as possible. Please write down your daily activities in the diary below, E.g. 'sitting', 'walking' (and if walking, whether up a hill or on the flat), and when you have gone to bed. If you feel any symptoms such as chest pain, palpitations, dizziness, or breathlessness please document this with the specific time.

Diary – Date _____ Time monitor put on _____

_9.00am_____

_10.00am_____

_11.00am_____

_12.00pm_____

_1.00pm_____

__2.00pm_____

_3.00pm_____

_4.00pm_____

_5.00pm_____

_6.00pm_____

_7.00pm_____

_8.00pm_____

_9.00pm_____

_10.00pm_____

Appendix 2

Holter Monitoring Proforma

Patient sticker *Doctor seeing patient*_____

History *(To be filled in with patient when having monitor removed)*

IHD _____
(previous – heart attack, angioplasty, angina, bypass)

Arrhythmia _____
(Irregular heart beat, atrial fibrillation, pacemaker or other device)

Hypertension yes ☐ no ☐

If yes date diagnosed _____

Palpitations / Chest Pain / SOB / Syncope (circle)
_____
_____

Exercise tolerance _____ yards

NYHA class _____

Medication list
(Please write down all the medications you are taking currently and the dose)

Name_____Dose/Frequency_____

Class	Patient Symptoms
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

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