THE PATHOGENESIS, INVESTIGATION AND MANAGEMENT OF SYSTEMIC AMYLOIDOSIS

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

ABSTRACT

Background: Amyloidosis is a multisystem disorder characterised by abnormal protein folding, in which proteins adopt an abnormal conformation and deposit as insoluble fibrils that disrupt tissue structure and function.

Aims and Methods: To characterise the phenotype of the heredo-familial forms of systemic amyloidosis, the role of solid organ transplantation in the different systemic amyloidosis syndromes and to evaluate the gastroenterological and hepatological causes and sequaelae of systemic amyloidosis. These features were sought in patients followed at the UK National Amyloidosis Centre between 1984 and 2011.

Results and Conclusions: Familial amyloid polyneuropathy associated with the T60A transthyretin variant has a prominent cardiac phenotype, which negatively impacts upon prognosis. Furthermore this cardiac phenotype has a negative impact upon survival in those who undergo liver transplantation to eliminate variant transthyretin, which is synthesised in the liver, not only by increasing operative risk but also as there is likely ongoing cardiac amyloid deposition associated with wild-type transthyretin from the liver graft. In the face of failing organ function, liver and renal transplantation in hereditary lysozyme amyloidosis (ALys) appears feasible, despite ongoing amyloidogenesis and the risk of recurrent graft amyloid, due to slow turnover of amyloid. Similarly, renal and cardiac transplantation in AL amyloidosis and renal transplantation in AA amyloidosis appear favourable when strategies to suppress amyloidogenic precursor protein production are employed in tandem.

Gastrointestinal and hepatic amyloid are recognised in systemic amyloidosis. In ALys both are prevalent; the former is associated with endoscopic abnormalities and the latter may be asymptomatic or present as hepatic rupture. In patients diagnosed with amyloid by liver biopsy after presenting with deranged liver biochemistry, AL

amyloidosis is the commonest cause and although this presentation has been historically associated with a poor prognosis as there is frequently concomitant extra-hepatic amyloid, in those who achieve a good clonal response to chemotherapeutic regimes in the modern era of treatment a survival advantage is conferred. Primary gastrointestinal disease, such as inflammatory bowel disease, can cause systemic AA amyloidosis. Tight inflammatory control, guided by serial serum amyloid A protein levels, benefits amyloidotic kidneys, as does aggressive treatment of physiological renal stresses, such as sepsis. Systemic AL amyloidosis is commonly associated with malnutrition, especially when there is multisystem amyloid. Malnutrition in this group is associated with a poor quality of life and worse survival and it would appear therefore to be a potential target for intervention studies.

ETHICAL APPROVAL

All individuals who participated in the clinical research studies described in this thesis gave full informed consent in a format approved by the Royal Free Hospital Ethics Committee (REC Ref 06/Q0501/42). For the prospective study of malnutrition in systemic AL amyloidosis ethical approval was granted by the Riverside Ethics Committee (REC Ref 09/H0706/27). 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

Page

ABBREVIATIONS

AA	amyloid A protein amyloidosis
AApoAI	apolipoprotein AI amyloidosis
AApoAII	apolipoprotein AII amyloidosis
AApoAIV	apolipoprotein AIV amyloidosis
Aβ2M	β2 microglobulin amyloidosis
ACys	cystatin C amyloidosis
AEF	amyloid enhancing factor
AFib	fibrinogen A α-chain amyloidosis
AGel	gelsolin amyloidosis
AH	monoclonal immunoglobulin heavy chain derived amyloid
AL	monoclonal immunoglobulin light chain derived amyloid
ALP	alkaline phosphatase
ALys	lysozyme amyloidosis
ANS	autonomic neuropathy
AST	aspartate aminotransferase
ATTR	transthyretin amyloidosis
B_2M	Beta-2-microglobulin
BMI	body mass index
BNP	brain natriuretic peptide
CD	Crohn's disease
CKD	chronic kidney disease
CPHPC	R-1-[6-[R-2-carboxy-pyrrolidin-1-y1]-6-oxo-hexanoyl] pyrrolidine-2-carboxylix acid
CR	complete response

CRP	C-reactive protein
CV	coefficient of variance
DAB	3,3'-diaminobenzidine
DNA	deoxyribonucleic acid
ECG	electrocardiogram
ECOG	Eastern Co-Operative Group
eGFR	estimated glomerular filtration rate
ESRD	end stage renal failure
FAP	familial amyloid polyneuropathy
FLC	free immunoglobulin light chains
FCU	familial cold urticaria
FMF	familial Mediterranean fever
GI	gastrointestinal
GM-CSF	granulocyte macrophage colony-stimulating factor
HIDS	hyper immunoglobulin D syndrome
HR	hazard ratio
H_2O_2	hydrogen peroxide
IBD	inflammatory bowel disease
IFN-γ	interferon- γ
IL-1	interleukin 1
IL-6	interleukin 6
IQR	inter-quartile range
IVSd	intraventricular septal thickness in diastole
LECT-2	leucocyte cell-derived chemotaxin 2
LVPWd	left ventricular posterior wall thickness in diastole
MBq	megaBecquerel

MGUS	monoclonal gammopathy of undetermined significance
mSv	millisieverts
MWS	Muckle-Wells syndrome
N	number
NAC	National Amyloidosis Centre
NaCl	sodium chloride
NaOH	sodium hydroxide
NH_4CL	ammonium chloride
NICE	National Institute of Clinical Excellence
NR	no response
NT-proBNP	N-terminal prohormone brain natriuretic peptide
N-terminal	amino terminal
OLT	orthotopic liver transplant
PAP	peroxidase-anti-peroxidase
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PG-SGA	patient-generated subjective global assessment
PNS	peripheral neuropathy
PPI	proton pump inhibitor
PR	partial response
QOL	quality of life
RNA	ribonucleic acid
RRT	renal replacement therapy
RTx	renal transplant
SAA	serum amyloid A protein
SAP	serum amyloid P component

SCT	stem cell transplantation
TBS	Tris buffered saline
TNF- α	tumour necrosis factor - α
TRAPS	tumour necrosis factor associated periodic syndrome
TTR	transthyretin
UC	ulcerative colitis

LIST OF FIGURES

LIST OF TABLES

Chapter 1

Introduction

Amyloid Structure

Amyloidosis is a disorder characterised by abnormal protein folding, in which proteins adopt an abnormal conformation and deposit as insoluble fibrils that disrupt tissue structure and function. Amyloidosis can be acquired or hereditary and amyloid deposition can be localised or systemic. Over 20 different proteins are known to form amyloid fibrils in vivo, but not all cause overt disease. Amyloid is identified by the pathognomonic finding of apple-green dichroism when tissue is stained with the dye Congo red and visualised under cross-polarised light. The characteristic appearance of amyloid fibrils under the electron microscope is that of rigid non-branching fibrils with a diameter of ~10 nm.[1] Despite the heterogeneity of the precursor proteins which form amyloid fibrils, diffraction studies have highlighted that the structure and properties of all amyloid fibrils are remarkably similar and adopt a β-sheet structure.[2] The protofilament structure of amyloid fibrils is made up of ß-sheets, which run parallel to the axis of the protofilament, with their component ß-strands perpendicular to the fibril axis.[3]

Non-fibrillar constituents of amyloid deposits include glycosaminoglycans, proteoglycans and amyloid P component, which is identical to and derived from the normal plasma glycoprotein serum amyloid P component (SAP). Glycosaminoglycans are located primarily on the surface of cells in the extracellular matrix and consist of negatively charged unbranched polysaccharides containing a repeated disaccharide unit. Though universal in all amyloid deposits, their role is unclear. Heparan sulphate

proteoglycans are also a universal component of amyloid deposits, which are thought to contribute to and maintain the conformational changes required for amyloid formation. Serum amyloid P component is another non-fibrillar constituent and is a member of the pentraxin family of plasma proteins.[4] Its physiological role has not been fully clarified but it may be important in host defence[5] or the development of autoimmunity.[6] It is present in all amyloid deposits and has calcium-dependant binding motifs. It is resistant to proteolysis and in-vitro binding of SAP to amyloid fibrils prevents degradation of amyloid by phagocytic cells and proteolytic enzymes.[7] In amyloidosis circulating SAP exists in a dynamic equilibrium with the SAP bound to amyloid fibrils and only SAP molecules in the circulation undergo catabolism. Its role in amyloidogenesis has been proven by the inability to induce experimental AA amyloidosis in SAP knockout mice.[8]

Pathogenesis of amyloidosis

Amyloidosis is characterised by aberrant folding of various precursor proteins.[9] The pathogenesis of amyloid revolves around "off-pathway" folding of the various fibril precursor proteins into a ß-sheet structure with highly organised auto-aggregration as amyloid fibrils.[10] Precursor protein homozygosity, such as SAA1 isotypes in AA amyloidosis[11] and variant TTR[12] promotes susceptibility to and severity of amyloidosis. Amino acid sequences of relevant proteins may dictate the potential of a protein to form amyloid on the background of a sustained supply of fibril precursor protein but the genetic and environmental factors that confer susceptibility to amyloid formation are not fully understood.[13] Furthermore the factors that govern pattern of organ involvement and clinical manifestations are also not fully appreciated. This is apparent both between and within different types of amyloidosis to such a degree that there can be major phenotypic differences between members of a kindred with the same genetic mutation that encodes for a particular hereditary form of systemic amyloidosis. In

experimental mice models injection of amyloidotic tissue acts as a primer to develop AA amyloidosis within days of receiving an acute phase inflammatory stimulus in contrast to unprimed mice where there is a requirement of an inflammatory stimulus for many weeks.[14] The amyloid fibrils themselves have been identified as the "amyloid-enhancing factor" (AEF) and increase the propensity of precursor protein in forming amyloid possibly by forming a template upon which continued supply of precursor protein can deposit in an exponential manner.[15]

The pathological effects of amyloid are secondary to extracellular deposition of amyloid with disruption of tissue architecture and subsequent impairment of organ function. There is some evidence of a cytotoxic effect of amyloid fibrils perhaps through apoptosis.[16] This may account for the fact that the clinical and investigative parameters suggestive of organ damage may not correlate with body amyloid load or amyloid infiltration of affected organs. The rate of amyloid deposition may also be a factor with potential time for functional compensation in amyloidotic organs in those where the turnover of amyloid is slower.[17]

Amyloid degradation

Left untreated the natural history of amyloidosis is one of progressive symptomatology, organ failure and death. With treatments that eliminate or suppress the amyloidogenic precursor proteins one can see regression of amyloid from infiltrated organs.[18] This is presumably because the balance between amyloid formation and deposition versus regression swings in favour of the latter.[1] It has been postulated that macrophages may have a role in amyloid regression. Infiltration of amyloid deposits by macrophages is followed by the formation of multinucleate giant cells, which surround, engulf and destroy the amyloid. This has been highlighted in vivo by macrophage depletion with liposomal clodronate,[19] which has been shown to retard amyloid regression. Conversely

macrophage activation by GM-CSF (Pepys MB, unpublished data) can promote amyloid clearance. It is quite possible that the differences in rates of regression of amyloid are secondary to differences in phenotype and function of macrophages or monocytes, as much as it is dependent on suppressing amyloidogenic precursor protein production. The net effect of amyloid regression is stabilisation or improvement of organ function coupled with increased patient survival.

Classification of systemic amyloidosis is based on the respective fibril precursor protein (Table 1.1). Amyloid deposition may occur in several different situations – firstly, when there is a prolonged abnormally high concentration of a structurally normal, but weakly amyloidogenic protein e.g., Serum Amyloid A protein (SAA) in chronic inflammatory states or ß2-microglobulin in long-term end-stage renal failure; secondly, in the presence of an abnormal protein with inherently amyloidogenic potential e.g., certain monoclonal immunoglobulin light chains or variant proteins encoded by genetic mutations; and finally over a very long period when there is a normal concentration of a normal protein with weak amyloidogenic potential such as in senile systemic amyloidosis in which wild-type transthyretin accumulates as amyloid in the hearts of elderly patients. The pattern of clinical features in systemic amyloidosis depends upon which organs are involved and sometimes suggests a particular amyloid type. Studies in hereditary systemic amyloidosis have revealed a correlation between the phenotype and the size of the precursor protein fragment that is incorporated into the amyloid fibrils. This may provide insights into the pathogenetic mechanisms in other forms of amyloidosis.[20]

Table 1.1 Classification of systemic amyloidosis.

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^{21}	Monoclonal immunoglobulin heavy chains	Systemic amyloidosis associated with monoclonal plasma cell dyscrasias
Aβ2M	β2-microglobulin	Systemic amyloidosis associated with long-term dialysis Dominant musculoskeletal symptoms
ATTR	Normal plasma transthyretin	Senile systemic amyloidosis with prominent cardiac involvement
ATTR	Genetically variant transthyretin	Autosomal dominant systemic amyloidosis Familial amyloid polyneuropathy or hereditary amyloid cardiomyopathy
ALys	Genetically variant lysozyme	Autosomal dominant systemic amyloidosis Non-neuropathic with prominent visceral involvement
AGel	Genetically variant gelsolin	Autosomal dominant systemic amyloidosis Cranial nerve involvement with lattice corneal dystrophy
ACys	Genetically variant cystatin C	Hereditary cerebral haemorrhage with cerebral and systemic amyloidosis of Icelandic type
AApoAI	Genetically variant apolipoprotein AI	Autosomal dominant systemic amyloidosis Predominantly non-neuropathic with prominent visceral involvement
AApoAII	Genetically variant apolipoprotein AII	Autosomal dominant systemic amyloidosis Non-neuropathic with prominent renal involvement
AApoAIV	Wild-type apolipoprotein AIV	Sporadic systemic amyloidosis associated with aging.
AFib	Genetically variant fibrinogen A α-chain	Autosomal dominant systemic amyloidosis Non-neuropathic with prominent renal involvement

Clinical Amyloidosis Syndromes

Systemic AL Amyloidosis

This is the commonest form of amyloidosis in the developed world and is associated with an underlying clonal dyscrasia of plasma cells or B-lymphoid/ lymphoplasmacytoid cells, which may otherwise be benign.[22] The age-adjusted incidence in the USA is 8.9 per million person-years.[23] Approximately 2% of individuals with monoclonal gammopathies (MGUS) develop AL amyloidosis.[23] The fibrils are formed from the N-terminal domain of monoclonal kappa or lambda immunoglobulin light chains, more commonly lambda than kappa, and consist of the whole or part of the variable (VL) domain, although intact light chains are sometimes present. There is increasing evidence from sequence analyses of Bence-Jones proteins of both kappa and lambda type from patients with AL amyloidosis that these variable region polypeptides contain unique amino acid substitutions or insertions compared to non-amyloidogenic monoclonal light chains.[24] The deletion 13q and ploidy changes occur more frequently in AL amyloidosis than MGUS or myeloma.[25] Certain light chain variable region (VL) genes are over-represented in AL amyloidosis and may have an impact on organ tropism.[26] A monoclonal protein can be identified by serum or urine electrophoresis in 65% and 86% of AL patients respectively.[22] A monoclonal excess of FLC can be identified at diagnosis in 98% of patients.[27]

Potentially, all organs can be directly affected by amyloid deposits besides the brain. The symptoms at onset can be non-specific and include malaise and weight loss. Kidney involvement presents as proteinuria or renal impairment. Cardiac amyloid usually manifests as a restrictive cardiomyopathy; typically there is concentric ventricular wall thickening resulting in diastolic dysfunction and impaired left ventricular filling resulting in congestive cardiac failure. Low voltage complexes on

electrocardiogram in the context of these echocardiographic changes are strongly suggestive of cardiac amyloid. Prognosis is poor with cardiac amyloid and median survival is 4-6 months after appearance of congestive cardiac failure.[28] Cardiac biomarkers are useful in the diagnostic process and also provide prognostic information.[29] Diastolic dysfunction and increased genetic expression of natriuretic peptide genes in the amyloid-infiltrated ventricles leads to an increase in plasma B-type natriuretic peptide (BNP) levels.[30] A 30% reduction in N-terminal prohormone brain natriuretic peptide, which is co-secreted with BNP, after 3 doses of chemotherapy has been associated with improved survival.[31] Autonomic nervous system involvement presents variably as orthostatic hypotension, impotence, urinary retention and with gastrointestinal dysfunction and also confers a poor prognosis.[32] In those with a peripheral neuropathy there is a distal sensory deficit which can progress to a motor neuropathy in more advanced cases. There are a plethora of potential soft tissue features in AL amyloidosis including pseudohypertrophy of skeletal muscle, arthropathy, cutaneous lesions, carpal tunnel syndrome, periorbital bruising, macroglossia, lymphadenopathy and nail dystrophy.[33] Bleeding diatheses such as factor X and Factor IX deficiency are also recognised.[34] Hepatomegaly is common and may result from amyloid infiltration or be secondary to congestive cardiac failure or both. The typical serum biochemical picture in hepatic amyloidosis is elevation in the alkaline phosphatase and gamma-glutamyl transferase, related to sinusoidal infiltration.[35] Occasionally liver failure may ensue. Gastrointestinal tract involvement may cause motility disturbances (often secondary to autonomic neuropathy), malabsorption, altered bowel habit and gastrointestinal haemorrhage.[36]

Systemic AA Amyloidosis

AA amyloidosis may occur in association with any kind of chronic inflammatory disorder. The lifetime incidence of AA amyloidosis in those with chronic inflammatory conditions is 1-5%.[37] In the developed world the most frequent predisposing conditions are the inflammatory arthritides, followed by chronic sepsis, inflammatory bowel disease and the hereditary periodic fever syndromes. There are 5 forms of inherited periodic fever syndromes: familial Mediterranean fever (FMF), hyper-immunoglobulin D syndrome (HIDS), TNF receptor associated periodic fever syndrome (TRAPS), familial cold urticaria (FCU) and Muckle-Wells syndrome (MWS). FMF is the commonest form. It is inherited in an autosomal recessive manner and is characterised by recurrent self-limiting attacks of fever, serositis and sometimes arthritis or rash.[38] It is prevalent in the Eastern Mediterranean. Tuberculosis still remains a major cause of AA amyloidosis in the developing world. Six percent of patients with AA amyloidosis have no clinically overt inflammatory disease.[39] The amyloid precursor protein is the N-terminal fragment of the acute phase reactant SAA, an apolipoprotein constituent of high-density lipoprotein. SAA is synthesised by hepatocytes and its gene transcription is regulated by cytokines, in particular IL-1 and IL-6.[40] SAA concentration may rise 1000-fold during inflammation and remain persistently elevated until the inflammation remits. Although the median latency between onset of inflammation and diagnosis is 8 to 14 years some individuals develop amyloid within months. Outcome in AA amyloidosis is favourable when SAA concentration remains below 10mg/l.[41] Nephrotic syndrome and/or renal impairment are common modes of presentation with approximately ¼ cases progressing to end-stage renal failure. Liver involvement in AA amyloidosis is a feature of advanced disease and confers a poor prognosis.[42] An amyloid cardiomyopathy and autonomic neuropathy are rare.[39]

Dialysis-associated/β2-microglobulin amyloidosis

β2-microglobulin amyloidosis (Aβ2M) occurs in patients receiving long-term dialysis for end-stage renal failure and predominantly affects bones and joints. β2M, which is the amyloidogenic precursor protein, is a non-glycosylated protein found in all nucleated cells that is normally metabolised in the kidney. It is freely filtered at the glomerulus and then reabsorbed and catabolised by the proximal tubular cells.[43] In Aβ2M, which occurs both in haemodialysis and peritoneal dialysis populations, β2M concentration in the blood is much increased.[44] The clinical features include carpal tunnel syndrome, arthralgia of the shoulders, knees, wrists and small joints with associated swelling, tenosynovitis and occasionally haemarthroses.[45] Subchondral erosions and cysts can contribute to pathological fractures. Gastrointestinal manifestations are rare but have been reported.[36] The incidence of Aβ2M is falling presumably because of newer dialysis membranes, cleaner dialysis fluids and higher-flux dialysis.[1]

Hereditary systemic amyloidosis

There are several forms of hereditary systemic amyloidosis, which are associated with genetically variant proteins, mostly comprising single amino acid substitutions. They are inherited in an autosomal dominant manner with variable penetrance accounting for a frequent absence of a family history. Age of onset, mode of presentation, pattern of organ involvement and rate of progression vary even within affected kindreds suggesting a contributory role of other factors. Historically, there has been a division into neuropathic and non-neuropathic forms. Familial amyloid polyneuropathy (FAP), which encompasses the former, is the commonest type (Table 1.1).

Familial amyloid polyneuropathy

Patients with familial amyloid polyneuropathy possess a mutation in their transthyretin (TTR) gene. TTR is synthesised predominantly in the liver. It is a tetrameric protein responsible for transport of thyroxine and retinol-binding protein. The commonest encoding mutation is a valine-for-methionine substitution at position 30 (V30M). FAP was first described in 1952 in Portugese kindreds,[46] affected members of which were later discovered to be heterozygous for the TTR mutation encoding the V30M variant.[47] Well-described foci occur in Sweden,[48] Japan,[49]and Portugal.[50] Overall, more than 100 pathogenic mutations have been described. It is estimated that there are somewhere in the region of 10000 affected cases (http://www.foldrx.com). Tetramers of transthyretin, containing amyloidogenic variants, dissociate into monomers and form amyloid more readily than the wild-type protein. Typical symptoms of familial amyloid polyneuropathy include progressive peripheral and autonomic neuropathy along with cardiac amyloid, all of which can result in profound wasting and malnutrition that usually leads to death within 9-13 years.[51] In some countries, such as Sweden and Japan, a bimodal age distribution is recognised with a different phenotype and prognosis between the two age groups.[52] Other potential manifestations include renal involvement,[53] vitreous amyloid secondary to variant transthyretin derived from the retina and choroid plexus[54] and oculoleptomeningeal amyloid deposition manifesting as encephalopathy, dementia, seizures and a radiculopathy.[55]

Hereditary non-neuropathic systemic amyloidosis

The non-neuropathic forms of hereditary systemic amyloidosis were first described by Ostertag in 1932.[56] They are derived from variants of apolipoprotein AI (although this may occasionally cause neuropathy), apolipoprotein AII, lysozyme and fibrinogen A-α chain.[57] A renal phenotype is dominant but cardiac amyloidosis, gastrointestinal

haemorrhage, soft tissue amyloidosis and even hepatic rupture are recognised depending upon the underlying amyloidogenic variant (Table 1.1).

Senile Systemic Amyloidosis

This is caused by deposition of amyloid derived from wild-type TTR, which is produced in the liver.[58] The dominant clinical manifestations are related to cardiac amyloid (presenting as congestive cardiac failure or conduction disturbances), although carpal tunnel syndrome is common. Senile systemic amyloidosis is usually confined to individuals over the age of 70 years and the cardiac features typically progress slowly. It is frequently mistaken for cardiac failure secondary to coronary artery disease. Subclinical gastrointestinal tract involvement, typically detected histologically within subserosal veins, is present in 41% of individuals with senile systemic amyloidosis aged over 80 years.[59]

Localised amyloidosis

Localised amyloidosis represents amyloid confined to a single organ or tissue which will not evolve into systemic amyloidosis. In a series of 208 patients at the UK National Amyloidosis Centre (NAC) the commonest organ systems involved by localised amyloidosis were the urinary and respiratory tracts (unpublished data). Localised amyloidosis is thought to evolve from monoclonal light chains secreted by a localised focus of abnormal plasma cells. Evidence for this comes from immunohistochemical analyses of amyloid coupled with molecular techniques and gene re-arrangement studies.[60] Follow-up studies have revealed no evolution into systemic amyloidosis up to 20 years after diagnosis.[61] It should however be borne in mind that localised amyloid may also be an early manifestation of systemic AL amyloidosis with the potential for other organ involvement at a later time. For this reason, careful

follow-up of patients who are thought to have localised amyloidosis is needed,[62] particularly in the presence of a plasma cell dyscrasia. Differentiating between localised and systemic amyloidosis is important since systemic chemotherapy is rarely required in localised amyloidosis.

Diagnosis of Amyloidosis

Histology

The diagnosis of amyloidosis is often made at a late stage, long after onset of symptoms due to its non-specific and varied presentations that can mimic many other conditions. Diagnosis requires histological confirmation of amyloid and is achieved by staining amyloidotic tissue with Congo-red and observing the pathognomonic red-green birefringence under cross-polarised light visualised with light microscopy.[63] Common biopsy sites include the gastrointestinal tract, kidneys and bone marrow. A screening rectal biopsy remains the commonest means of obtaining a histological diagnosis of amyloid from the gastrointestinal tract when systemic amyloidosis is suspected due to the ease of the technique and an acceptable sensitivity of between 75-94%.[64] Abdominal fat aspiration is another means of acquiring histological confirmation of amyloid. Liver biopsy via a percutaneous route is not recommended if amyloid is suspected because of haemorrhagic risk.[65] Amyloid is commonly an incidental and unexpected biopsy finding which should trigger further investigations to determine the precise amyloid fibril type and extent of organ involvement.

Immunohistochemistry to determine the amyloid fibril type should routinely be performed in any patient with amyloid but despite recent advances in immunohistochemical techniques, often remains inconclusive.[66] Distinguishing between AL and hereditary amyloidosis by clinical manifestations and

immunohistochemistry alone may not be possible. Monoclonal gammopathies are not infrequent in the general population, and although suggestive, the presence of a monoclonal gammopathy in a patient with amyloidosis does not prove AL-type and other investigations are required before a definitive diagnosis can be made. Additional screening for mutations in the genes encoding known amyloid fibril proteins may be necessary to exclude hereditary forms of amyloidosis.[67] Precise identification of the amyloid fibril type is critical since treatment is type-specific.

SAP scintigraphy

Another diagnostic method, which is available in a few specialised units, is SAP scintigraphy. SAP binds to all amyloid fibrils. When it is radiolabeled with iodine as a nuclear medicine tracer it can be utilised to quantify visceral amyloid deposits in vivo.[68] SAP scintigraphy can be used to monitor regression or progression of visceral amyloid and response to treatment.[18] Furthermore, the distribution of visceral amyloid deposits may be a strong indicator of amyloid type.[69] In AA amyloidosis, hepatic involvement is associated with a poor prognosis[42] and total body amyloid load by SAP scintigraphy is critical in evaluating the risk/benefit ratio of treatments such as stem cell transplantation for AL amyloidosis.[1]

Cardiac investigations

The standard means of assessing cardiac amyloidosis is by echocardiography. The characteristic findings are ventricular wall thickening, diastolic dysfunction and bi-atrial dilatation with relative sparing of systolic function until advanced stages of disease. Another corroborative feature is low voltage complexes on electrocardiogram, which contrasts the increased voltages that are typical of ventricular wall thickening due to hypertension. Cardiac magnetic resonance imaging is a newer investigative modality

which shows promise. Cardiac biomarkers have also proven very useful in studies of AL amyloidosis. Troponin T and N-Terminal pro B-type Natriuretic Peptide can stratify patients in terms of survival and prognosis.[29]

Other investigations

Determining and monitoring the concentration of amyloidogenic precursors, such as monoclonal immunoglobulin light chains in AL amyloidosis and SAA in AA amyloidosis, is helpful diagnostically and is critical to determine the adequacy of treatment. Serum free light chain concentration can now be measured by sensitive nephelometric immunoassay (Freelite) for a free light chain excess and should be accompanied by serum and urine electrophoresis, a bone marrow aspirate and a skeletal survey to characterise the underlying clonal disorder. Genetic sequencing is very useful as hereditary forms of amyloidosis can occur in those without a family history due to the poor penetrance of the encoding mutations.[67]

Treatment of amyloidosis

Treatment of localised amyloidosis is via surgical resection, but only if symptomatic. Treatment of the systemic amyloidosis syndromes is more complicated and a two-fold approach should be pursued. The universal aim, in the absence of amyloid-specific therapy, is reduction of the supply of the respective amyloid fibril precursor protein (Table 1.2) but meticulous organ support is frequently also needed. Dialysis is useful in end-stage renal failure with approximately ¼ of patients (249 patients) with renal amyloid of AL-type requiring it during the course of the disease during follow-up at the NAC, with a median survival of 39 months from dialysis.[70] Approximately 40% of

Table 1.2 Treatment: reduction or elimination of the supply of fibril precursors in systemic amyloidosis.

Disease	Aim of treatment	Example of treatment
AA amyloidosis	Suppress acute phase response and thereby production of SAA	Anti-inflammatory and immunosuppressive therapy in rheumatoid arthritis and Crohn's disease (e.g. anti-tumour necrosis factor) Colchicine for familial Mediterranean fever
AL amyloidosis	Suppress production of monoclonal immunoglobulin light chains	Chemotherapy or stem cell transplantation directed at plasma cell dyscrasias
Hereditary amyloidosis	Reduce/eliminate source of genetically variant protein	Orthotopic liver transplantation for familial amyloid polyneuropathy secondary to variant transthyretin or for selected cases of fibrinogen A α-chain and apolipoprotein AI amyloidosis.
Haemodialysis amyloidosis	Reduce plasma concentration of β_2-microglobulin	Renal transplantation

patients with AA amyloidosis (158 patients), who have been followed up at the NAC have required dialysis with a median subsequent survival of 46 months. The survival on dialysis in AA amyloidosis is comparable to non-diabetic causes of end-stage renal failure.[71] Solid organ transplantation (heart, kidney or liver) to keep patients alive long enough to reap the benefits of reducing the relevant fibril precursor is another potential strategy.

Reduction or elimination of the production of the amyloidogenic precursor protein may lead to regression of amyloid deposits and stabilisation or improvement in amyloidotic organ function. Initial therapeutic efficacy is monitored in terms of reduction of precursor protein supply, which may be followed by improvement in amyloidotic organ function and accompanied by amyloid regression on SAP scintigraphy. Importantly, regression of amyloid from organs does not always translate into recovery of function and conversely, recovery of amyloidotic organ function is not always accompanied by regression of deposits (they may remain stable). Accumulation of amyloid however, almost always leads to deterioration in organ function. Determining and monitoring the serial concentrations of amyloidogenic precursor proteins, such as monoclonal immunoglobulin light chains in AL amyloidosis and SAA in AA amyloidosis, is extremely important in determining the adequacy of and guiding treatment.

AL amyloidosis

The prognosis of systemic AL amyloidosis has improved but there are still major challenges.[72] The Italian amyloidosis group have reported a median survival of 46 months in 705 patients visiting their centre recently.[73] Poor prognostic factors include cardiac amyloid,[29] autonomic neuropathy,[32] jaundice[35] and multisystem disease.[27] Treatment revolves around suppression of the underlying B-cell clone and therefore

production of amyloidogenic serum free light chains, which are the precursor proteins. The treatment regimes in AL amyloidosis have been adapted from those used in multiple myeloma, although there are important differences between treatment of myeloma and AL amyloidosis. The plasma cell dyscrasias that underlie cases of AL amyloidosis tend to be subtle and low-grade, a complete haematological response is not the universal goal in AL amyloidosis since a partial response may be sufficient to lead to improved organ function,[27] and toxicity of therapy is often substantial in patients with amyloidosis whose amyloidotic organs may have reduced functional reserve. Regression of amyloid is a gradual process, which may lead to measurable clinical improvement of organ function months or even years after successful suppression of the underlying clonal disorder.[74] Mobilisation of amyloid from organs such as the heart is much slower than from the kidneys and the liver.[75]

The choice of treatments is between chemotherapy with oral alkylators, intermediate dose chemotherapy, stem cell transplantation (SCT) or newer chemotherapeutic agents such as lenalidomide and bortezomib.[72] The first chemotherapeutic regime adopted for AL amyloidosis was melphalan and prednisolone with slow responses in only about 20% of patients.[76] In the United Kingdom intermediate dose chemotherapy regimes are used, such as cyclophosphamide, thalidomide and dexamethasone where clonal responses are achieved in 74%[77] or melphalan and dexamethasone where clonal responses are achieved in 67%.[78] Bortezomib is a proteosome inhibitor and has a licence as a second-line agent in those with inadequate clonal responses to a prior line of treatment under NICE guidance for myeloma with amyloidosis as a myeloma-defining criterion according to International Myeloma Working Group classification.[79] In one multi-centre study of bortezomib clonal responses were achieved in 71% within 2 months of treatment including complete clonal responses in 25%.[80] It has the added attraction of being efficacious

without the need for concomitant steroid administration and therefore avoids the associated side-effects of the latter. Lenalidomide has a licence as a third line agent under NICE guidance with observed clonal response rates in up to 67%.[81] SCT can be offered to candidates with a good performance status and limited organ involvement. Although SCT is probably associated with the highest chance of a complete and sustained clonal response it is also associated with a significant chance (4-43%) of treatment-related mortality in patients with AL amyloidosis. Careful patient selection is therefore needed and a favoured approach in the UK targets those without overt cardiac amyloid, no history of gastrointestinal haemorrhage, two or less vital organ involvement by amyloid and an age below 65 years. There has been one small prospective randomised study comparing SCT with combination chemotherapy and the findings were not definitive.[82]

AA amyloidosis

In AA amyloidosis the aim is to reduce production of SAA. Patient survival, amyloidotic organ function and change in amyloid load are directly influenced by SAA concentration with regression of amyloid in 60% of cases in a series of 374 patients, when sustained normal SAA values of <10 mg/l were achieved.[41] Prognosis was dependent upon the degree of renal dysfunction at diagnosis of amyloidosis and age of diagnosis.[39] As well as standard anti-inflammatory therapies, newer biologic agents that target pivotal inflammatory cytokines (e.g. TNFα and IL-1) have had an immense impact on the treatment of a variety of conditions that underlie AA amyloidosis including the inflammatory arthritides, Crohn's disease and the hereditary periodic fever syndromes. In the case of Castleman's disease surgical excision of the lymphoid tissue producing IL-6 has a similar effect.[83] In the case of familial Mediterranean fever (FMF), early use of colchicine usually prevents development of AA amyloidosis by

preventing inflammation; in established cases of AA amyloidosis due to FMF, colchicine can prevent ongoing amyloid accumulation.

Familial amyloid polyneuropathy

The treatment of hereditary systemic amyloidosis in cases where the amyloidogenic precursor protein is produced by the liver has been revolutionised by liver transplantation, which provides a form of "surgical gene therapy". It has been used most successfully in familial amyloid polyneuropathy.[84] Transplantation results in rapid and near total replacement of the variant protein by donor wild-type TTR, since almost all circulating TTR is produced in the liver.[85] If done at an early symptomatic stage of neuropathy then there is potential for improvement in survival and also stabilisation or even improvement of neuropathy.[86] This has been seen in those with the V30M variants endemic to Portugal, Sweden and Japan. In other variants where there might be cardiac amyloid this survival advantage may be lost because of the phenomenon of a paradoxical accelerated amyloid cardiomyopathy, where wild-type transthyretin deposits as amyloid on the template of cardiac amyloid secondary to variant transthyretin. This happens in an accelerated manner resulting in progressive cardiac failure.[87] Evidence for this has come from cardiac biopsy studies where there is an increase in the ratio of wild-type transthyretin compared to variant transthyretin in the amyloid fibrils of hearts of patients who have had liver transplants compared to those who have not.[88] A potential inadvertent benefit of liver transplantation in this patient group is that the explanted liver can be a "domino" donor organ.[89] The domino liver may be a source of variant transthyretin but is macroscopically and functionally normal. However there is a very small risk of "de novo" amyloid in domino graft recipients, which has been reported in a few case reports.[90] Liver transplantation has also been used in other forms of hereditary systemic amyloidosis – fibrinogen A-α chain

amyloidosis and apolipoprotein AI amyloidosis – where the precursor protein is wholly or partially derived from the liver respectively.[91,92] In those where transplantation is not an option symptomatic management has a role in those with cardiac complications, malnutrition, bladder and bowel dysfunction.[93]

Dialysis-related amyloidosis

The only effective treatment for dialysis-related amyloidosis is renal transplantation.[94] If undertaken there is a fall in serum levels of ß2–microglobulin accompanied by a rapid improvement in symptoms. There can be regression of amyloid on serial SAP scintigraphy but at a slower rate than the improvement in symptomatology. The anti-inflammatory properties of immunosuppression may also have a contributory role to this symptom resolution.[95] Bone cysts tend to persist for many years and heal slowly.[96] The incidence of Aβ2M amyloidosis is falling presumably because of improvements in dialysis.[1] Drug treatments including non-steroidal anti-inflammatory analgesics and steroids are not particularly effective.[97]

Future prospects in treatment

The mainstay of treatment in systemic amyloidosis currently revolves around reduction of the abundance of the relevant amyloid precursor protein. More recently however, studies have focused on targeting different stages of the amyloidogenesis pathway, usually downstream from precursor protein production.

Stabilisation of protein precursors is one potential therapeutic strategy. This is being evaluated for transthyretin (TTR), which is weakly amyloidogenic in its wild-type form and more amyloidogenic in its variant forms which cause familial amyloid polyneuropathy. Mutations render the tetrameric TTR protein less stable, allowing it to dissociate into monomeric components which can undergo conformational changes to

form amyloid fibrils. The physiological role of TTR is to carry thyroxine and retinol-binding protein and its binding sites are well characterised. Studies investigating drugs bound by TTR are in progress, including that of diflunisal, a non-steroidal anti-inflammatory drug that stabilises TTR in vitro.[98] A phase II/III study of diflunisal in familial amyloid polyneuropathy is currently underway. Iodinisation of candidate drugs such as diflunisal may also confer benefit by producing higher affinity binding. This concept was put forward after the newfound appreciation of the unique physiological properties of iodine atoms in thyroxine, which allows thyroxine to bind to TTR tightly in a stable conformation.[99] Another TTR stabiliser which is currently being evaluated in a phase II/III trial in patients with familial amyloid polyneuropathy is Fx-1006A (FoldRx). Although precursor protein stabilisation to date has focused on TTR, the principles could be applied to other types of amyloidosis. Another potential therapeutic area in TTR-related amyloidosis is the targeting of hepatic synthesis of TTR with small interfering RNA's, antisense oligonucleotides and single-strand oligonucleotides.[100]

Inhibiting fibrillogenesis is another potential treatment strategy. The antimicrobial doxycycline has been found in transgenic mouse models of familial amyloid polyneuropathy to disrupt TTR amyloid fibrils[101] and is currently being studied in a phase II clinical study. Heparan sulphate proteoglycans are a universal component of amyloid deposits, which are thought to contribute to and maintain the conformational changes required for amyloid formation. Analogues of N-acetylglucosamine inhibit binding of heparan sulphate and amyloid fibrils and orally administered low molecular weight sulphated molecules can inhibit development of experimentally induced AA amyloid in murine models.[102] Further evidence for the role of heparan sulphate in amyloidogenesis comes from experiments with transgenic mice that overexpress human heparanase, an enzyme that degrades heparan sulphate. They appear to be resistant to

the experimental induction of AA amyloidosis in certain murine organs.[103] Subsequent to these findings a phase II/III clinical trial was undertaken comparing eprodisate (Kiacta, Neurochem), a low molecular weight analogue of heparan sulphate, and placebo in 183 patients followed up over 24 months. Compared to placebo, eprodisate significantly reduced the risk of doubling of serum creatinine ($p=0.02$), increased the chance of a 50% reduction in creatinine ($p=0.01$) and favourably altered the slope of decline in creatinine clearance ($p=0.02$). Progression to end-stage renal failure and risk of death were not significantly affected however.[104]

Another potential treatment strategy that merits exploration is immunotherapy. Amyloid is relatively inert in vivo and exempt from immunological responses, but is nonetheless a good immunological target as amyloid fibrils, irrespective of the contributing precursor protein, have similar structural properties and epitopes that antibodies can target.[105] Administered antibodies have induced amyloid regression in murine models of both AA and AL amyloidosis.[106]

The efforts of the NAC have been focused on targeting serum amyloid P component, the non-fibrillar plasma glycoprotein, which is present in all amyloid deposits. Its role in amyloidogenesis was proven by the inability to induce experimental AA amyloidosis in SAP knockout mice.[8] These results have led to the development in the laboratories at the NAC of a drug, R-1-[6-[R-2-carboxy-pyrrolidin-1-y1]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid (CPHPC), which inhibits the binding of SAP to amyloid fibrils. It has a palindromic structure and cross-links SAP leading to its rapid clearance by the liver.[107] Initial results from an open-label study suggest that there may be attenuation of amyloid deposition rather than amyloid regression. However using a combination of CPHPC and anti-human SAP antibodies in human SAP transgenic mice there has been success in

inducing regression of amyloid.[108] This novel approach is set to be translated into the clinical arena in collaboration with GlaxoSmithKline.

Aims and Scope of the Thesis

The introduction to this thesis gives an outline of the current understanding of the pathogenesis, investigation and management of the different forms of systemic amyloidosis.

The subsequent studies cover three broad themes, which perfuse throughout the thesis. The first revolves around characterisation of the phenotype of the heredo-familial forms of systemic amyloidosis; the second provides an analysis of the role of solid organ transplantation in the different forms of systemic amyloidosis; the third theme focuses on the assessment and treatment of the gastroenterological and hepatological aspects of the systemic amyloidosis syndromes.

Solid organ transplantation has historically been stigmatised against in systemic amyloidosis because of the multisystem nature of the disease and furthermore there have been concerns about potential recurrence of amyloid in transplanted grafts. To evaluate its role, there is firstly review of the role of liver transplantation, as a form of surgical gene therapy, in familial amyloid polyneuropathy (FAP) associated with the T60A TTR variant, which is the commonest form of FAP in the UK. To date there has been scant data on its role outside of its use in the more common FAP V30M population. There is also a focus on the effect of cardiac amyloid, which is more common in FAP T60A than in FAP V30M, on prognosis in both a transplanted and non-transplanted FAP T60A population. There is subsequently assessment of the role of solid organ transplantation for amyloidotic organ failure in several forms of systemic amyloidosis throughout the thesis; liver and kidney transplantation in hereditary lysozyme amyloidosis (ALys), which currently has no anti-amyloid therapy and in which, therefore, there is risk of amyloid recurrence in grafts; liver, kidney and heart transplantation in the acquired forms of systemic amyloidosis (AL and AA). For these latter conditions, which have therapeutic options to suppress amyloid formation,

potential algorithms are proposed encompassing a tandem approach of solid organ transplantation together with amyloid-specific treatment strategies.

The gastrointestinal and hepatological sequaelae of systemic amyloidosis, though common, are less studied than several of the other potential complications. A review of patients diagnosed with amyloidosis unexpectedly by liver biopsy highlights phenotypic, prognostic and treatment differences between the different amyloidosis types and provides opportunity to propose a diagnostic and treatment algorithm should systemic amyloidosis present in this manner. There is also commentary on the prevalent combination of gastrointestinal and hepatological complications associated with the extremely heterogeneous ALys. Gastrointestinal disease as a potential primary cause of systemic amyloidosis is emphasised in the analysis of systemic AA amyloidosis associated with inflammatory bowel disease. There is focus on risk factors for disease development, analysis of progression of amyloidosis and suggestion of potential treatment goals. There is also a prospective study of nutritional status in AL amyloidosis, which is one of the largest prospective studies performed in systemic amyloidosis to date. Weight loss, though appreciated as a prevalent entity in this patient group, has gained little attention. Disease-specific factors associated with malnutrition are identified and the effects of malnutrition on quality of life and survival are assessed.

Chapter Two

Materials and Methods

Declaration

The concepts behind all of the studies, collection and analysis of data, recruitment and assessment of patients in the prospective study of nutritional status in systemic AL amyloidosis were all carried out by myself, as a clinical research fellow at the National Amyloidosis Centre, University College Medical School (Royal Free Campus), except where indicated below:

Measurements for biochemical and haematological data were performed by the hospital and departmental laboratory service.

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

Histological and immunohistochemical analysis was performed by Janet Gilbertson and Toby Hunt and laser microdissection and mass-spectrometry based proteomic analysis on one specimen was performed by Ahmet Dogan (Mayo Clinic, Rochester, USA).

Echocardiography was performed by Babita Pawarova and Carolyn McCarthy.

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

Data for the Canadian patients with familial amyloid polyneuropathy (T60A) was obtained from Dr. Angelika Hahn from the Department of Clinical Neurological Sciences, the University of Western Ontario, Canada and data for the Swedish patients with familial amyloid polyneuropathy (V30M) was provided by Professor Ole Suhr and Dr. Sadahisa Okamoto from Umea, Sweden.

There was assistance with the statistics in the thesis from Zoe Fox from the Department of Population Sciences, Department of Medicine, UCL and Aviva Petrie from the Biostatistics Unit at UCL Eastman Dental Institute.

Patients

All of the patients in this thesis were seen at the UK National Amyloidosis Centre, except for three patients, who were seen at the Department of Clinical Neurological Sciences, University of Western Ontario, Canada. In the 3-year period between 2008 and 2010, during my studentship, there were approximately 8000 patient consultations in the centre of which nearly 25% were new referrals. An access database in the centre has details of all of these patients, as well as other patients seen at the NAC over a period of approximately 20 years.

Histology

Congo-red staining

Amyloid deposits stained with Congo-red give pathognomonic red-green birefringence when viewed under cross-polarised light and this remains the gold-standard diagnostic method for amyloid.[63] This birefringence is dependent on a sufficient density of amyloid so formalin-fixed deparaffinised tissue sections were cut at 6-8 μm rather than

the usual 2-3 μm. Before staining, the sections were rehydrated, counterstained with haematoxylin, 'blued' under running tap water, rinsed in pure water and then stained by a modified version of the alkaline-alcoholic Congo-red method described by Puchtler et al. (1962).[63] This method reduces any non-specific background staining. The sections were then dehydrated through a series of ascending ethanol concentrations to xylene and mounted in DPX mounting medium. Slides were then viewed under brightfield and high-intensity polarised light microscopy and the presence and extent of amyloid deposition noted. Positive controls were obtained from a known Congo-red positive composite block validated by laser microdissection and mass-spectrometry based proteomic analysis and were always processed in parallel.

Typing of amyloid by immunohistochemistry

Congo-red staining of histology was followed by immunohistochemical staining of formalin-fixed deparaffinised sections of amyloidotic tissue to further characterise the amyloid type. Immunohistochemical staining of the amyloid deposits was performed using a panel of anti-human monospecific antibodies reactive with SAA (Eurodiagnostica), TTR, lysozyme, kappa and lambda immunoglobulin light chains (Dako Ltd, Denmark House, Ely, UK), apolipoprotien A1 (Genzyme Diagnostics) and fibrinogen Aα-chain (Calbiochem). For TTR staining, pre-treatment was performed for maximum antigen retrieval (10 minute incubation with 1% sodium periodate, wash, 10 minute incubation with 0.1% sodium metabisulphate, wash, 5-hour incubation at room temperature with 6M Guanadine in 0.9% sodium chloride). Duplicate sections were stained with each antibody +/- Congo red overlay to classify amyloid type.

For immunohistochemical analysis sections were taken to water and endogenous peroxidase activity was quenched by 30 minute incubation in aqueous (0.3%) hydrogen peroxide (H_2O_2). They were then rinsed in phosphate-buffered saline (PBS) containing

0.05% Tween (Calbiochem). Prior to application of the primary antisera, non-specific tissue binding was abolished by 30 min incubation in normal non-immune serum from the species providing the secondary antibody (Vector Part of the ImmPRESS Kit). Sections were incubated overnight with primary antisera at 4ºC. They were then rinsed with PBS containing 0.05% Tween (Calbiochem) and labelled with secondary antibodies from the appropriate part of the ImmPRESS Kit. Sections were washed in PBS and bound enzyme-antibody complexes were visualised using a metal-enhanced DAB (Fisher Scientific solution). Congo red overlay was also used in duplicate sections. After a brief rinse in pure water, sections with immunohistochemical staining were counterstained in haematoxylin, 'blued' under running tap water and stained with Congo-red. Absorption controls were used when possible by incubating the primary antibody with its targeted antigen overnight at 4ºC prior to incubation with the tissue.

Laser microdissection and mass-spectrometry based proteomic analysis

This was performed in one patient with Leucocyte cell-derived chemotaxin 2 (LECT-2) amyloidosis at the Mayo Clinic, USA.[109] Ten μm sections of liver tissue were stained with Congo-red and then the amyloid deposits underwent laser microdissection. Microdissected fragments were digested with the proteolytic enzyme trypsin, the tryptic peptides were separated by reverse-phase high performance liquid chromatography and the peptides were analysed by electrospray tandem mass spectrometry (MS). MS raw data files of molecular mass and peptide sequence were collated and compared against a database of proteins using different search engines such as MASCOT, which identified proteins present in the liver by using probability-based algorithms.

SAP Scintigraphy

SAP scintigraphy is a sensitive and specific means of imaging amyloid deposits in vivo,[110] with localisation of radiolabeled SAP to amyloid deposits in proportion to the amyloid present in viscera. This quantitative technique therefore allows serial monitoring of amyloid load and its change. Each subject undergoing SAP scintigraphy received by intravenous injection approximately 200μg of SAP bearing approximately 190 MBq of ^{123}I corresponding to an effective equivalent of 3.8 mSV, which is comparable to that from an intravenous urogram. Thyroid uptake was blocked by administration of 60mg potassium iodide just before the study and 5 further doses over the course of 3 days. Anterior and posterior whole body imaging was performed in each individual 6 or 24 hours after injection of ^{123}I-SAP by means of an IGE-Starcam gamma-camera (IGE Medical Systems, Slough, UK). Serial SAP scintigraphy was performed at 6-monthly or annual visits to gauge change in amyloid load.

The whole body amyloid burden was classified as 0 when there was no abnormal localisation of tracer; as small when uptake in one or more organs was visible but the intensity of the blood-pool background signal remained normal; as moderate when abnormal uptake in one or more organs was sufficiently intense for the blood-pool background signal to be partially lost when the grey-scale was normalised to encompass the target organ signal; and as large when the blood-pool background was lost with adjustment of the grey scale to encompass the target organ uptake. On serial scans regression of amyloid was defined as a reduction in tracer uptake in affected organs or an increase in the blood-pool background signal, or both; and accumulation of amyloid was defined as an increase in tracer uptake in affected organs, an abnormal tracer uptake in a previously unaffected organ, or a decrease in the blood-pool background signal; and a stable amyloid burden was defined as unchanged tracer localisation.

Cardiac assessment

Cardiac amyloid is poorly visualised by SAP scintigraphy. This is probably because of cardiac motility and a high blood-pool signal from the cardiac chambers. Cardiac amyloidosis is better evaluated therefore with electrocardiography and echocardiography.[29] Echocardiography was performed in all patients with two-dimensional and M-mode settings using a GE Vivid 7 system. Views of the heart were obtained from the parasternal long axis and the apical long axis positions. The presence of left ventricular wall thickening, left ventricular diastolic dysfunction, left ventricular systolic dysfunction (ejection fraction and fractional shortening) and left atrial diameter were measured using criteria defined by the British Society of Echocardiography (http://www.bsecho.org) and left atrial area was measured using criteria defined by the American Society of Echocardiography (http://www.asecho.org). The classical electrocardiographic features of low voltage QRS complexes, pathological "Q" waves (pseudo-infarct pattern) in the antero-septal leads and conduction abnormalities were recorded in all patients. Limb lead low-voltage amplitude was defined by a mean QRS amplitude in leads I, II, III, aVL and aVF of less than 0.5 mV and in the chest leads by the sum of the S wave in lead V1 plus the mean of the R wave in leads V5 or V6 being less than 1.5 mV.[111] Both electrocardiography and echocardiography were repeated at 6-monthly or annual visits.

Cardiac biomarkers have been increasingly used in the assessment of cardiac amyloid and in the provision of prognostic information.[31] N-terminus pro-B Natriuretic Peptide (NT-pro BNP) and Troponin T are two such biomarkers. Their concentrations were measured at 6-monthly or annual intervals in patients. Measurements were carried out using an electrochemiluminescence sandwich immunoassay (ECLIA, Roche) on a ROCHE Modular Analytics E170 platform. Serum NT-proBNP levels are typically higher in women and increase with age, therefore upper reference limits (the 97.5

percentiles of healthy subjects) in men and women are, respectively, 10.1 pmol/L and 15.3 pmol/L in subjects <50 years old; and 24.8 pmol/L and 33.9 pmol/L in individuals >50 years old (data generated by Roche from 712 normal subjects). Within-run precision was determined to be 3.5% at 23.9 pmol/L (22 samples) and 2.6% at 1318 pmol/L (22 samples); between-run precision was 3.9% at 25 pmol/L (22 samples) and 3.1% at 1403.3 pmol/L (22 samples). The detection limit reported by the manufacturer is 0.6 pmol/L. The working range of the Troponin T assay is 0.003-10µg/l. Troponin T within-run precision was determined to be 3.2% at 0.02µg/l (17 samples) and 1.3% at 2.1 µg/l (18 samples); between-run precision was 3.7% at 0.02µg/l (17 samples) and 2.2% at 2.2µg/l (17 samples).

The Mayo staging system is a prognostic tool in AL amyloidosis using an amalgamation of Troponin T and NT-proBNP values. It was designed with the understanding that cardiac amyloid is common in AL amyloidosis and that it has a significant bearing on survival in patients with AL amyloidosis. Cardiac biomarkers are considered more sensitive and specific than echocardiography in the evaluation of cardiac amyloid, as the interpretation of the latter may be confounded by factors such as hypertension and co-existing coronary artery disease. Furthermore the relatively slow rate of change of cardiac amyloid on echocardiography makes it less attractive as a prognostic tool. Dispenzieri et al. therefore developed the Mayo staging system. Mayo stage I classifies patients with a NT-proBNP value <332ng/L and Troponin T <0.035µg/l, stage II represents patients in whom one marker is below this threshold and stage III classifies patients in whom neither value is below this threshold.[112] Their study found significant differences in patient survival based upon this simple prognostic scoring system with worst survival in those with Mayo stage III disease.

Renal Assessment

Renal function was classified according to CKD staging (Table 2.1).[113] It is a validated scoring system, which, irrespective of cause of renal disease, takes into account the fact that renal function may be normal or even increased in patients with damaged kidneys (i.e. eGFR>60 ml/min) and that in this population there is an increased risk of progressive loss of kidney function and furthermore an increased morbidity and mortality risk, in particular cardiovascular mortality. This is very pertinent to patients with renal amyloid where proteinuric renal dysfunction frequently occurs in the presence of an eGFR>60 ml/min.

Criteria for diagnosis of amyloid-related major organ involvement

Organ involvement in patients with systemic amyloidosis was defined according to abnormal uptake on SAP scintigraphy and according to the international amyloid consensus criteria (Table 2.2)[114] in those with AL amyloidosis and in the patients with familial amyloid polyneuropathy (T60A). The latter criteria relies on abnormalities in investigations in the cases of renal, cardiac, hepatic, gastrointestinal or pulmonary amyloid, but relies on clinical examination for diagnosis of an amyloid neuropathy and soft tissue amyloidosis, as outlined in Table 2.2. An amyloidotic peripheral neuropathy presents as a distal small fibre sensory dysfunction, which in more advanced stages progresses to a motor neuropathy and the autonomic neuropathy presents with symptoms of gastrointestinal dysmotility or pseudo-obstruction, urinary voiding dysfunction and frequently postural hypotension and, in men, impotence.

Table 2.1 Stages of CKD (Chronic kidney disease).

Stage	**eGFR (ml/min)**	**Description**
1	90+	Normal kidney function but urine findings or structural abnormalities or genetic trait point to kidney disease
2	60-89	Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease
3	30-59	Moderately reduced kidney function
4	15-29	Severely reduced kidney function
5	<15 or on dialysis	Very severe or end-stage kidney failure

Table 2.2 International amyloid consensus criteria for organ involvement in AL amyloidosis.

Organ involved	**Criteria**
Heart	Echocardiography : Mean wall thickness >12 mm, no other cardiac cause
Kidneys	24-hour urine protein >0.5g/day, predominantly albumin
Liver	Total liver span >15cm in the absence of heart failure or alkaline phosphatase >1.5 times upper limit of normal
Peripheral neuropathy	Clinical symmetrical lower extremity sensorimotor peripheral neuropathy
Autonomic neuropathy	Gastric emptying disorder, pseudo-obstruction, Voiding dysfunction not related to direct organ Infiltration
Gastrointestinal tract	Direct biopsy verification with symptoms
Lung	Direct biopsy verification with symptoms Interstitial radiographic pattern
Soft tissue	Tongue enlargement, clinical arthropathy, claudication of vascular origin, skin, myopathy by biopsy or pseudohypertrophy, lymph node, carpal tunnel syndrome

Performance status

Performance status is an attempt to quantify cancer patients' general well-being and level of function with respect to daily activities. It is a useful guide to determine whether they can receive treatment, whether dose adjustment is needed or whether palliation would be more suitable. The Eastern Co-operative Group (ECOG) performance status (Table 2.3)[115] was conceived by ECOG, which was established in 1955 as a co-operative to run multi-centre cancer clinical trials in the USA. This measure of performance status has been utilised in studies of patients with a variety of cancers and has also been used in patients with systemic AL amyloidosis.[116] It was used in the study of solid organ transplantation in AL amyloidosis in this thesis (chapter 7)

Immunoassays

C-reactive protein (CRP)

CRP was measured in the serum using a high-sensitivity automated microparticle-enhanced latex turbidimetric immunoassay (COBAS MIRA; Roche Diagnostics GmbH).[117] The lower limit of detection was 0.2 mg/l with an inter-assay coefficient of variance (CV) of 4.2% at 4 mg/l and 6.3% at 1 mg/l.

Serum amyloid A protein (SAA)

SAA was measured in serum by latex nephelometry (BNII autoanalyser; Dade Behring, Marburg, Germany).[118] The lower limit of detection was 0.7 mg/l, with an inter-assay CV of 2.6% at 15 mg/l and 3.7% at 80 mg/l. Standardisation of both CRP and SAA assays was based on the respective WHO International Reference Standards (WHO 1987).[119]

Table 2.3 Eastern Co-operative Group classification.

Grade	Summary	Description
0	Normal	Able to carry out normal activities without restriction
1	With effort	Restricted in physical strenuous activity Ambulatory, can do light work
2	Restricted	Ambulatory and capable of self-care Unable to carry out work
3	Dependant	Capable of only limited self-care Confined to bed or chair for more than 50% of waking hours
4	Immobile	Cannot carry out any self-care; Totally confined to bed or chair

Serum free immunoglobulin light chain assay

Serum kappa and lambda free immunoglobulin light chains (FLC) were measured using a latex-enhanced immunoassay (The Binding Site, Birmingham, UK) on a Behring BNII autoanalyser (Dade Behring, Marburg, Germany).[120-122] The assay utilises antibodies directed against FLC epitopes that are hidden in whole immunoglobulin molecules, and has a sensitivity of < 5 mg/l. This compares with typical detection limits of 150-500 mg/l by immunofixation, and 500-2000 mg/l by electrophoresis. The reference range was established using 100 healthy blood donor sera in which the mean concentrations of polyclonal free kappa and free lambda light chains were respectively, 11.38 mg/l (95% confidence intervals, 7.41-16.77 mg/l) and 17.36 mg/l (95% confidence intervals, 8.91-29.87 mg/l), with a mean kappa to lambda ratio of 0.7 (95% confidence intervals, 0.37-0.95). Monoclonal FLC was identified as values for kappa or lambda that exceeded the respective reference ranges and produced an abnormal kappa to lambda ratio.

Gene sequencing

Genomic DNA was isolated by a rapid method[123] from frozen whole blood taken into EDTA. The blood was added to NH_4CL and the sample spun down, resuspended in 0.9% NaCl, spun down and resuspended in 0.05M NaOH. This was then incubated, cooled and neutralised with 1M Tris pH8. The coding regions of the transthyretin (exons 2, 3 and 4), apolipoprotein AI (exons 3 and 4) and fibrinogen A α-chain (exon 5) genes were amplified by the polymerase chain reaction (PCR) using Ready-To-Go tubes (GE Healthcare) and the lysozyme gene (exon 2) was amplified by PCR using the HotStarTaq DNA Polymerase kit (Qiagen). The primers used as part of the PCR process are outlined in Appendix 1.

Study procedures specific to the study of nutritional status in AL amyloidosis

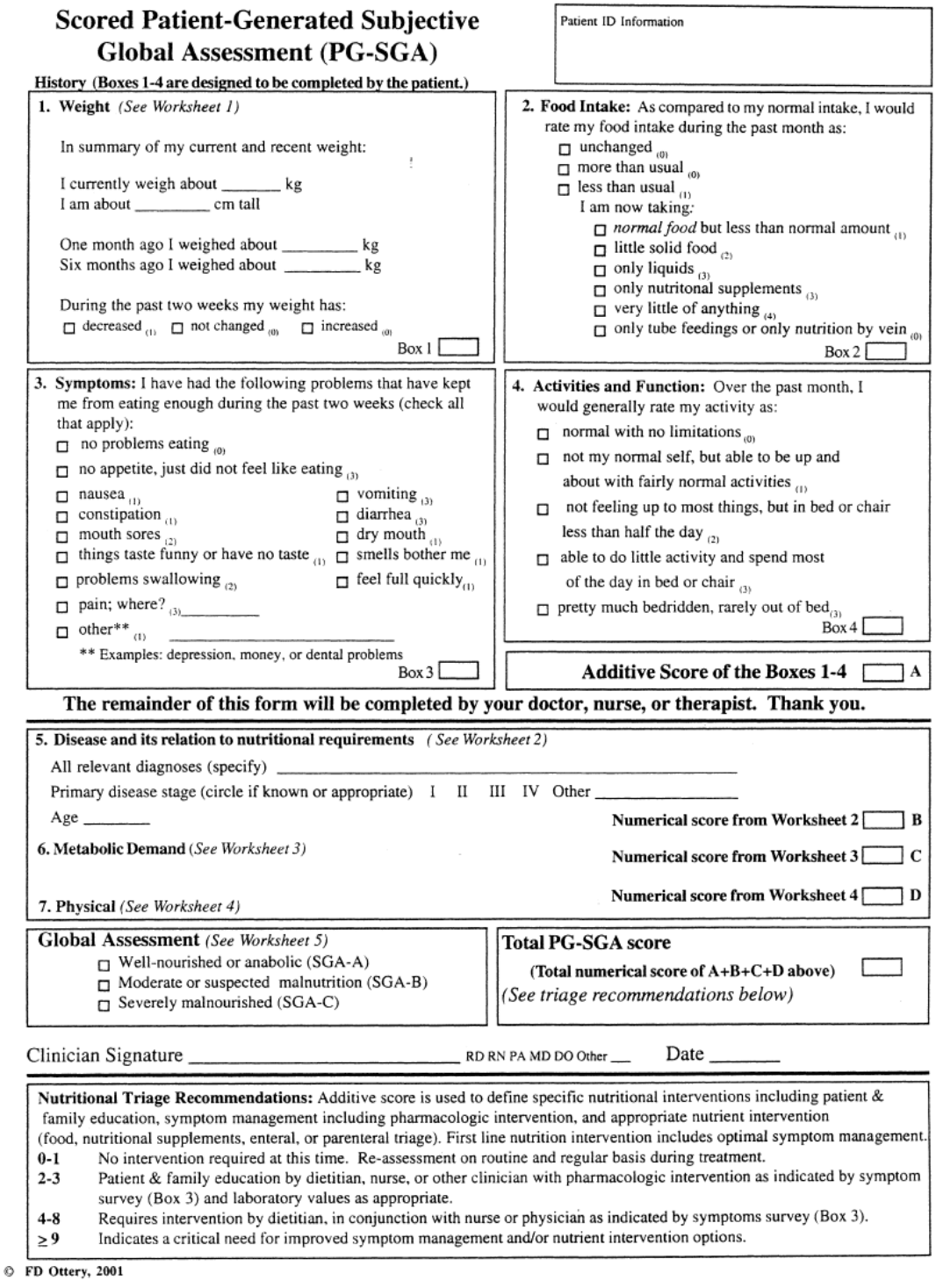
Nutritional status assessment

The patient–generated subjective global assessment (PG-SGA) was used to characterise nutritional status in treatment-naïve patients with AL amyloidosis in chapter 5 (Figure 2.1). It was initially intended for use in cancer patients[124] but has since been applied to other patient groups, such as stroke patients.[125] It consists of a check-box questionnaire with a section to be completed by the patient and a section to be completed by the clinician resulting in a score of 0 to 35 with higher scores related to greater malnourishment. The four medical components (weight loss, nutrition impact symptoms, intake and functional capacity) are completed by the patient. The assessing clinician completes a second part to the form (diagnosis, age and metabolic stress) and also conducts a physical examination assessing fat, muscle stores and fluid status. The PG-SGA has been shown to have a high degree of inter-rater reproducibility and high sensitivity and specificity when compared with other validated nutrition tools.[124]

Quality of life assessment

Quality of life (QOL) was assessed by the EORTC QLQ-C30 questionnaire,[126] which is composed of both multi-item scales and single-item measures (Figure 2.2). It includes 5 functional scales, 3 symptom scales, a global health status/QOL scale and 6 single symptom items. Raw scoring is achieved by averaging the items in a given scale and a linear transformation standardises the raw score into a score ranging from 0-100. High scores for a symptom scale/item represent a high level of symptomatology, high scores in a functional scale represent a high level of functioning and high scores in global

Figure 2.1 Scored Patient-Generated Subjective Global Assessment (1) (PG-SGA)



Scored Patient-Generated Subjective Global Assessment (PG-SGA)

Patient ID Information

History (Boxes 1-4 are designed to be completed by the patient.)

1. Weight *(See Worksheet 1)*

In summary of my current and recent weight:

I currently weigh about _____ kg
I am about _____ cm tall

One month ago I weighed about _____ kg
Six months ago I weighed about _____ kg

During the past two weeks my weight has:
☐ decreased (1) ☐ not changed (0) ☐ increased (0)

Box 1 ☐

2. Food Intake: As compared to my normal intake, I would rate my food intake during the past month as:
☐ unchanged (0)
☐ more than usual (0)
☐ less than usual (1)
I am now taking:
☐ *normal food* but less than normal amount (1)
☐ little solid food (2)
☐ only liquids (3)
☐ only nutritonal supplements (3)
☐ very little of anything (4)
☐ only tube feedings or only nutrition by vein (0)

Box 2 ☐

3. Symptoms: I have had the following problems that have kept me from eating enough during the past two weeks (check all that apply):
☐ no problems eating (0)
☐ no appetite, just did not feel like eating (3)
☐ nausea (1)
☐ constipation (1)
☐ mouth sores (2)
☐ things taste funny or have no taste (1)
☐ problems swallowing (2)
☐ vomiting (3)
☐ diarrhea (3)
☐ dry mouth (1)
☐ smells bother me (1)
☐ feel full quickly (1)
☐ pain; where? (3)_____
☐ other** (1) _____
** Examples: depression, money, or dental problems

Box 3 ☐

4. Activities and Function: Over the past month, I would generally rate my activity as:
☐ normal with no limitations (0)
☐ not my normal self, but able to be up and about with fairly normal activities (1)
☐ not feeling up to most things, but in bed or chair less than half the day (2)
☐ able to do little activity and spend most of the day in bed or chair (3)
☐ pretty much bedridden, rarely out of bed (3)

Box 4 ☐

Additive Score of the Boxes 1-4 ☐ **A**

The remainder of this form will be completed by your doctor, nurse, or therapist. Thank you.

5. Disease and its relation to nutritional requirements *(See Worksheet 2)*
All relevant diagnoses (specify) _____
Primary disease stage (circle if known or appropriate) I II III IV Other _____
Age _____

Numerical score from Worksheet 2 ☐ **B**

6. Metabolic Demand *(See Worksheet 3)*

Numerical score from Worksheet 3 ☐ **C**

7. Physical *(See Worksheet 4)*

Numerical score from Worksheet 4 ☐ **D**

Global Assessment *(See Worksheet 5)*
☐ Well-nourished or anabolic (SGA-A)
☐ Moderate or suspected malnutrition (SGA-B)
☐ Severely malnourished (SGA-C)

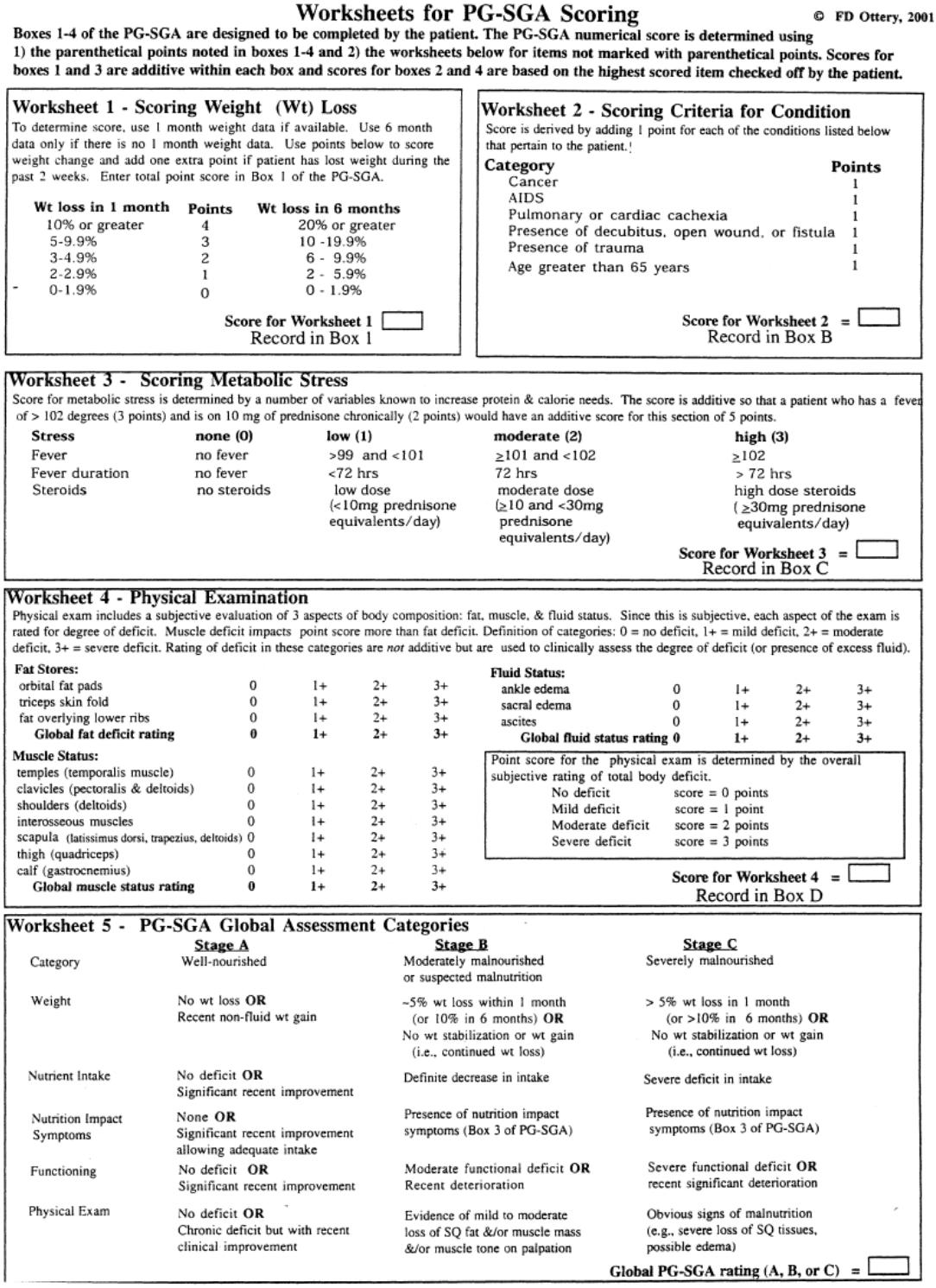
Total PG-SGA score
(Total numerical score of A+B+C+D above) ☐
(See triage recommendations below)

Clinician Signature _____ RD RN PA MD DO Other __ Date _____

Nutritional Triage Recommendations: Additive score is used to define specific nutritional interventions including patient & family education, symptom management including pharmacologic intervention, and appropriate nutrient intervention (food, nutritional supplements, enteral, or parenteral triage). First line nutrition intervention includes optimal symptom management.

0-1	No intervention required at this time. Re-assessment on routine and regular basis during treatment.
2-3	Patient & family education by dietitian, nurse, or other clinician with pharmacologic intervention as indicated by symptom survey (Box 3) and laboratory values as appropriate.
4-8	Requires intervention by dietitian, in conjunction with nurse or physician as indicated by symptoms survey (Box 3).
≥ 9	Indicates a critical need for improved symptom management and/or nutrient intervention options.

Figure 2.1 Scored Patient-Generated Subjective Global Assessment (2) (PG-SGA)



Worksheets for PG-SGA Scoring

Boxes 1-4 of the PG-SGA are designed to be completed by the patient. The PG-SGA numerical score is determined using 1) the parenthetical points noted in boxes 1-4 and 2) the worksheets below for items not marked with parenthetical points. Scores for boxes 1 and 3 are additive within each box and scores for boxes 2 and 4 are based on the highest scored item checked off by the patient.

Worksheet 1 - Scoring Weight (Wt) Loss

To determine score, use 1 month weight data if available. Use 6 month data only if there is no 1 month weight data. Use points below to score weight change and add one extra point if patient has lost weight during the past 2 weeks. Enter total point score in Box 1 of the PG-SGA.

Wt loss in 1 month	Points	Wt loss in 6 months
10% or greater	4	20% or greater
5-9.9%	3	10 -19.9%
3-4.9%	2	6 - 9.9%
2-2.9%	1	2 - 5.9%
0-1.9%	0	0 - 1.9%

Score for Worksheet 1 ☐
Record in Box 1

Worksheet 2 - Scoring Criteria for Condition

Score is derived by adding 1 point for each of the conditions listed below that pertain to the patient.

Category	Points
Cancer	1
AIDS	1
Pulmonary or cardiac cachexia	1
Presence of decubitus, open wound, or fistula	1
Presence of trauma	1
Age greater than 65 years	1

Score for Worksheet 2 = ☐
Record in Box B

Worksheet 3 - Scoring Metabolic Stress

Score for metabolic stress is determined by a number of variables known to increase protein & calorie needs. The score is additive so that a patient who has a fever of > 102 degrees (3 points) and is on 10 mg of prednisone chronically (2 points) would have an additive score for this section of 5 points.

Stress	none (0)	low (1)	moderate (2)	high (3)
Fever	no fever	>99 and <101	≥101 and <102	≥102
Fever duration	no fever	<72 hrs	72 hrs	> 72 hrs
Steroids	no steroids	low dose (<10mg prednisone equivalents/day)	moderate dose (≥10 and <30mg prednisone equivalents/day)	high dose steroids (≥30mg prednisone equivalents/day)

Score for Worksheet 3 = ☐
Record in Box C

Worksheet 4 - Physical Examination

Physical exam includes a subjective evaluation of 3 aspects of body composition: fat, muscle, & fluid status. Since this is subjective, each aspect of the exam is rated for degree of deficit. Muscle deficit impacts point score more than fat deficit. Definition of categories: 0 = no deficit, 1+ = mild deficit, 2+ = moderate deficit, 3+ = severe deficit. Rating of deficit in these categories are *not* additive but are used to clinically assess the degree of deficit (or presence of excess fluid).

Fat Stores:				
orbital fat pads	0	1+	2+	3+
triceps skin fold	0	1+	2+	3+
fat overlying lower ribs	0	1+	2+	3+
Global fat deficit rating	**0**	**1+**	**2+**	**3+**

Muscle Status:				
temples (temporalis muscle)	0	1+	2+	3+
clavicles (pectoralis & deltoids)	0	1+	2+	3+
shoulders (deltoids)	0	1+	2+	3+
interosseous muscles	0	1+	2+	3+
scapula (latissimus dorsi, trapezius, deltoids)	0	1+	2+	3+
thigh (quadriceps)	0	1+	2+	3+
calf (gastrocnemius)	0	1+	2+	3+
Global muscle status rating	**0**	**1+**	**2+**	**3+**

Fluid Status:				
ankle edema	0	1+	2+	3+
sacral edema	0	1+	2+	3+
ascites	0	1+	2+	3+
Global fluid status rating	**0**	**1+**	**2+**	**3+**

Point score for the physical exam is determined by the overall subjective rating of total body deficit.

No deficit	score = 0 points
Mild deficit	score = 1 point
Moderate deficit	score = 2 points
Severe deficit	score = 3 points

Score for Worksheet 4 = ☐
Record in Box D

Worksheet 5 - PG-SGA Global Assessment Categories

Category	Stage A Well-nourished	Stage B Moderately malnourished or suspected malnutrition	Stage C Severely malnourished
Weight	No wt loss **OR** Recent non-fluid wt gain	~5% wt loss within 1 month (or 10% in 6 months) **OR** No wt stabilization or wt gain (i.e., continued wt loss)	> 5% wt loss in 1 month (or >10% in 6 months) **OR** No wt stabilization or wt gain (i.e., continued wt loss)
Nutrient Intake	No deficit **OR** Significant recent improvement	Definite decrease in intake	Severe deficit in intake
Nutrition Impact Symptoms	None **OR** Significant recent improvement allowing adequate intake	Presence of nutrition impact symptoms (Box 3 of PG-SGA)	Presence of nutrition impact symptoms (Box 3 of PG-SGA)
Functioning	No deficit **OR** Significant recent improvement	Moderate functional deficit **OR** Recent deterioration	Severe functional deficit **OR** recent significant deterioration
Physical Exam	No deficit **OR** Chronic deficit but with recent clinical improvement	Evidence of mild to moderate loss of SQ fat &/or muscle mass &/or muscle tone on palpation	Obvious signs of malnutrition (e.g., severe loss of SQ tissues, possible edema)

Global PG-SGA rating (A, B, or C) = ☐

Figure 2.2 EORTC QLQ-C30 (Version 3 MODIFIED)

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: _____

Your birthdate (Day, Month, Year): _____

Today's date (Day, Month, Year): 31 _____

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4

Please go on to the next page

Figure 2.2 EORTC QLQ-C30 (Version 3 MODIFIED)

During the past week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
16. Have you been constipated?	1	2	3	4
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

health status represent a high QOL.[127] Its use in AL amyloidosis has already been validated in a randomised prospective phase II trial of chemotherapy in AL amyloidosis - UKATT study.[128]

Statistical Analysis

Statistical tests were performed using Graphpad Prism (Version 4), SPSS v20 (IBM SPSS) and the Stata 10.2 software package (StataCorp). For the purpose of descriptive statistics data was expressed as a median (range) or median (interquartile range). Kaplan-Meier analyses were used in all of the studies to estimate survival and log-rank tests were used to compare the survival between different populations in the study of FAP T60A (chapter 3), the study of nutritional status in AL amyloidosis (chapter 5) and the study of solid organ transplantation in AL amyloidosis (chapter 7). Cox proportional hazards regression was used to investigate factors associated with overall survival of patients in the study of FAP T60A, the study of nutritional status in AL amyloidosis and in the study of liver biopsies revealing amyloid (chapter 8). The relationships between NT-proBNP and echocardiographic parameters (chapter 3) and between the PG-SGA score and amyloid-specific biochemical and clinical features (chapter 5) were evaluated by standard regression analysis. The Pearson correlation coefficient was determined to assess the linear relationship between the QOL scores and the PG-SGA score (chapter 5). A p-value <0.05 was considered to be significant in all statistical analyses, except chapter 3 where p-values <0.2 were retained in a forward stepwise survival analysis and chapter 5 where all factors which were of statistical significance ($p<0.10$) in univariable analyses were included in the multivariable analysis.

Chapter 3

Cardiac phenotype and clinical outcome of familial amyloid polyneuropathy secondary to variant transthyretin (T60A)

Introduction

Familial Amyloid Polyneuropathy (FAP) is the commonest form of hereditary systemic amyloidosis and is caused by mutations of the transthyretin (TTR) gene. Tetrameric TTR is synthesised predominantly in the liver and when it contains amyloidogenic variants it can present as FAP. The clinical presentation and disease course of FAP varies substantially not only between mutations but also amongst patients with the same mutation. In fact, amyloidogenic TTR mutations are variably penetrant such that some individuals may carry a mutation and never develop FAP.

The clinical features and natural history of FAP have been best characterised in the FAP V30M population.[46,129-131] Typical features in this population include a progressive small fibre neuropathy affecting peripheral and autonomic nerves. An amyloid cardiomyopathy, which is relatively uncommon in FAP V30M, may be a more prominent feature of FAP associated with other TTR mutations.[52]

Liver transplantation (OLT) is the only established treatment for FAP and provides a form of surgical gene therapy,[84] as previously described. Favourable outcomes including a survival benefit have been reported with OLT in patients with FAP V30M, particularly if the procedure is undertaken early in the disease course.[86] However OLT in non-V30M FAP cases with pre-existing cardiac amyloidosis, has been

associated with poor outcomes, due to apparent acceleration of cardiac amyloid accumulation following the procedure.[87]

FAP associated with the TTR T60A variant (FAP T60A) was first described in 1986 in an Irish family.[132] Although there is a major endemic focus in County Donegal in North-West Ireland,[133] where it has been estimated that 1% of the population possess the relevant TTR mutation,[134] FAP T60A has been identified widely around the world.[135]

Aims

This study aims to report the clinical presentation, in particular the cardiac phenotype, of FAP T60A in the largest series reported to date. Most published series of FAP are based around the neuropathic features[46,129-131] and the role of liver transplantation[86] in FAP V30M. Studies of the "non-V30M" FAP populations, which are considered to have a higher frequency of cardiac amyloid,[52] are lacking and the few small studies that exist frequently encompass different TTR mutations. Cardiac amyloid has a significant impact upon prognosis in AL amyloidosis[28] but its potential impact upon survival in a large cohort of FAP patients with the same mutation and a high frequency of cardiac amyloid has not formally been studied. Similarly, the role of liver transplantation in "non-Met30" FAP populations with cardiac amyloid, where there is a risk of a paradoxical accelerated amyloid cardiomyopathy,[87] has largely been studied in small series of FAP with mixed "non-Met30" TTR mutations and has not been looked at systematically in a population with a single "non-Met30" TTR mutation with comparison to a non-transplanted cohort with the same TTR mutation. The FAP (T60A) patients in this series provided a population to evaluate these two areas.

Methods

Patients

The study included all 60 patients with FAP T60A followed at the UK NAC and at the Department of Clinical Neurological Sciences, University of Western Ontario, Canada, who were first assessed between 1992, when a prospective clinical protocol was initiated, and December 2009. The genetic diagnosis was proven in all cases by gene sequencing. Immunohistochemistry was performed in all available biopsy specimens.

Patients attended for their initial diagnostic evaluation and were re-assessed at 6-monthly to annual intervals. At each evaluation, patients underwent a detailed clinical review and were scheduled for electrocardiography and echocardiography along with blood and urine biochemistry. Clinical review comprised history and examination including weight, supine and standing blood pressure and oxygen saturations. Biochemistry included tests of renal, hepatic and cardiac function. Additional investigations, including tests of autonomic and peripheral nerve function, were undertaken periodically to support clinical findings. Organ involvement by amyloid was defined according to the international amyloid consensus criteria, originally defined for AL amyloidosis.[114]

Survival analyses and statistics

Patient follow-up was censored in December 2009, and, in the single patient who was lost to follow-up, at his last clinic visit prior to this date. Kaplan-Meier analyses and Cox proportional hazards regression were used to investigate factors associated with overall survival of all patients in the cohort. Variables considered for inclusion in multivariate models were age, presence of peripheral neuropathy, presence of autonomic neuropathy, ejection fraction, grade of diastolic dysfunction, interventricular

septal (IVS) thickness, left ventricular posterior wall (LVPW) thickness, left atrial (LA) diameter, LA area and whether the patient experienced significant weight loss. Cut points for NT-proBNP were according to previously published data in AL amyloidosis[70] and were the median values in the cohort for remaining variables. The relationship between NT-proBNP and a number of echocardiographic parameters was evaluated by standard regression analysis. Analyses were performed using the Stata 10.2 software package (StataCorp). All reported p-values are two-sided. A p-value <0.05 was considered to be significant, although all results with a p-value <0.2 were retained in a forward stepwise survival analysis.

Results

Patients

Sixty Caucasian patients with FAP T60A were referred from a wide variety of clinical specialties; 18 from cardiologists, 8 from haematologists, 12 from neurologists, 6 from gastroenterologists, 3 from rheumatologists, 2 from nephrologists and 11 from a mixture of other specialities. Forty patients had Irish ancestry and 5 were Scottish. Only 22 cases (37%) had a definite family history of amyloidosis.

DNA analysis

Direct DNA sequencing of the TTR gene revealed that all 60 patients were heterozygous for the previously reported single base substitution in exon 3 encoding alanine at residue 60 of the native protein.[136] The remainder of the sequence was normal in all cases.

Histology

The histological diagnosis of amyloidosis was made by gastrointestinal tract biopsy in 25 cases (rectal in 16), by cardiac biopsy in 14 cases, by nerve biopsy in 4 cases and by biopsy from other sites in 9 cases. The amyloid in each case stained with antibodies against TTR (Figure 3.1). Histologic samples were not obtained in 8 patients who had typical clinical phenotypes, the T60A TTR variant, and characteristic echocardiographic and neurophysiological findings. Four of these eight patients had a family history of biopsy-proven FAP T60A, and none had a plasma cell dyscrasia which might have raised the possibility of AL-type amyloidosis. It was not deemed ethical to pursue biopsies in these 8 patients on various clinical grounds.

Five patients, 3 of whom had inappropriately received chemotherapy for presumed systemic AL amyloidosis prior to review at the NAC, had a detectable plasma cell dyscrasia that proved incidental when TTR-type amyloid was confirmed immunohistochemically.

Clinical course and outcome

Median (range) age of development of symptoms was 63 (45-78) years. Twenty-five of 60 (42%) patients presented with cardiac symptoms; 25/60 (42%) and 14/60 (23%) presented with autonomic and peripheral neuropathy respectively, which in 4 patients developed concomitantly. Median (range) delay from symptom onset to diagnosis of amyloidosis was 24 (2-132) months. Although 42% patients presented with cardiac symptoms, 56/58 patients had echocardiographic evidence of advanced cardiac amyloidosis at diagnosis (Figure 3.2), and cardiac amyloidosis became evident in the 2 remaining patients during follow-up. Echocardiographic parameters at diagnosis are outlined in Table 3.1. Increased left ventricular wall thickness and diastolic dysfunction were present at diagnosis in 93% and 97% of patients respectively and 57% also had

Figure 3.1 Cardiac biopsy from a patient with FAP T60A. The panel on the top shows extensive amorphous pink material following staining with Congo-red dye when viewed under brightfield light. The middle panel shows red-green birefringence, pathognomonic of amyloidosis, when the same section is viewed under cross-polarised light. The panel on the bottom shows immunohistochemical staining of the same patient's biopsy with a monoclonal anti-human TTR antibody confirming the presence of TTR within the amyloid deposits.

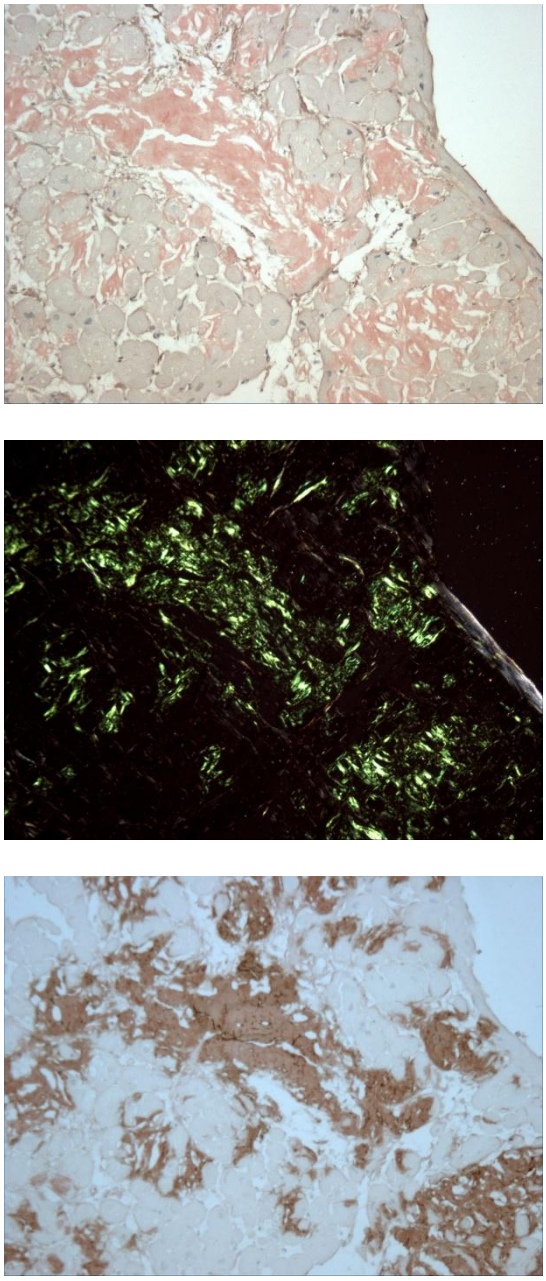
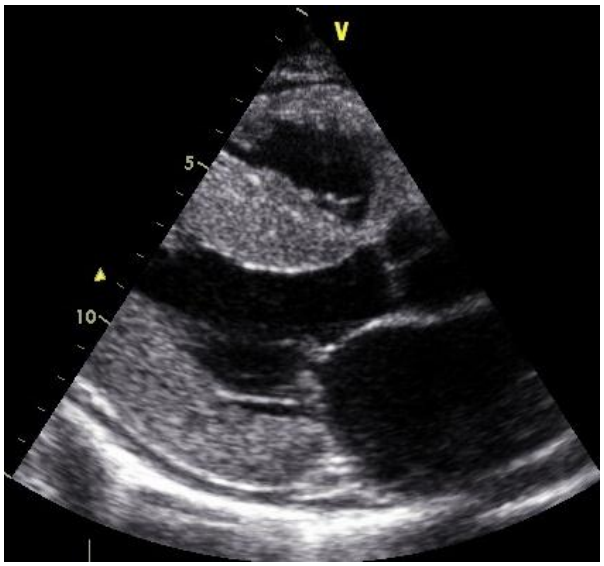


Figure 3.2 Echocardiographic investigations in a patient with FAP T60A. Parasternal long-axis (top) and two dimensional four-chamber view (bottom) showing enlarged atria, biventricular concentric wall thickening, thickened inter-atrial septum, thickened valves and a pericardial effusion.



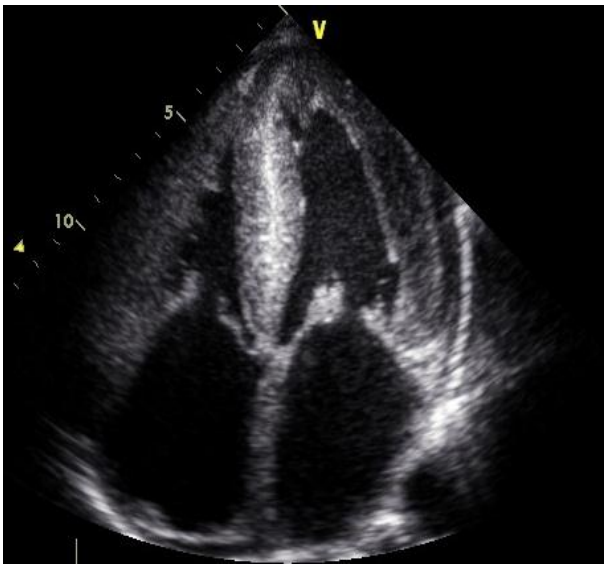


Table 3.1 Echocardiography at diagnosis in familial amyloid polyneuropathy associated with TTR T60A variant.

Echocardiography	Median (Range)	Normal values	Percentage Abnormal
Interventricular septal thickness in diastole (IVSd) (mm)	17 (10-25)	6-12	93
Left ventricular posterior wall thickness in diastole (LVPWd) (mm)	17 (11-25)	6-12	93
Diastolic dysfunction (Grade)	Grade II (0-IV)	0	97
Ejection fraction (EF) (%)	53 (22-73)	>55	57
Fractional shortening (%)	24 (10-40)	25-43	50
Left atrial diameter (LAd) (mm)	45 (36-52)	27-38	43
Left atrial area (cm^2)	21.1 (8.5-31.9)	<20	40

systolic dysfunction but only 12% had an ejection fraction <40%. Valve thickening was evident in 91% and pericardial effusions in 76% of cases.

Electrocardiographic abnormalities were evident in 53/56 (95%) cases at diagnosis. Only 7/44 patients with cardiac amyloid on echocardiography who had evaluable (i.e. non-paced) electrocardiograms had small QRS complexes, whereas the pseudo-infarction pattern of poor R wave progression in the chest leads was present in 25/44 (57%) patients (Figure 3.3).

Median (range) NT-proBNP concentration at diagnosis among 54 evaluable patients was 299 pmol/l (5-2146). Relationship between NT-proBNP and echocardiographic parameters is shown in Table 3.2. Higher NT-pro BNP was significantly and independently associated with both increased ventricular wall thickness and reducing LV ejection fraction but interestingly, was not associated with degree of diastolic dysfunction.

Neuropathic symptoms at diagnosis of FAP are shown in Table 3.3.

The cohort was followed for a median (range) of 31 months (0.4–132.0) from diagnosis. Progression of amyloidosis was observed during follow-up in all evaluable patients, in three-quarters of whom the cardiac and neuropathic features worsened simultaneously. Twenty-nine (48%) patients died at a median (range) age of 69 years (56-79). Median (95% CI) survival by Kaplan-Meier analysis among 52 non-transplanted FAP T60A patients from onset of symptoms was 6.6 years (95% CI: 6.1-9.8), and from diagnosis was 3.7 years (95% CI: 2.9-5.1), which was significantly worse than the median survival from symptom onset and diagnosis of 12.0 years (95% CI: 5.9-20.2) and 8.2 years (95% CI: 1.2-15.4) respectively in 26 Swedish FAP V30M patients, which represent a younger cohort with dominant neuropathic features (data provided by Professor O Suhr, Umea University, Sweden) (Figure 3.4). Survival was not

Figure 3.3 Electrocardiogram showing atrial fibrillation and pseudoinfarction pattern in the anterior chest leads with no attenuation of QRS complex size.

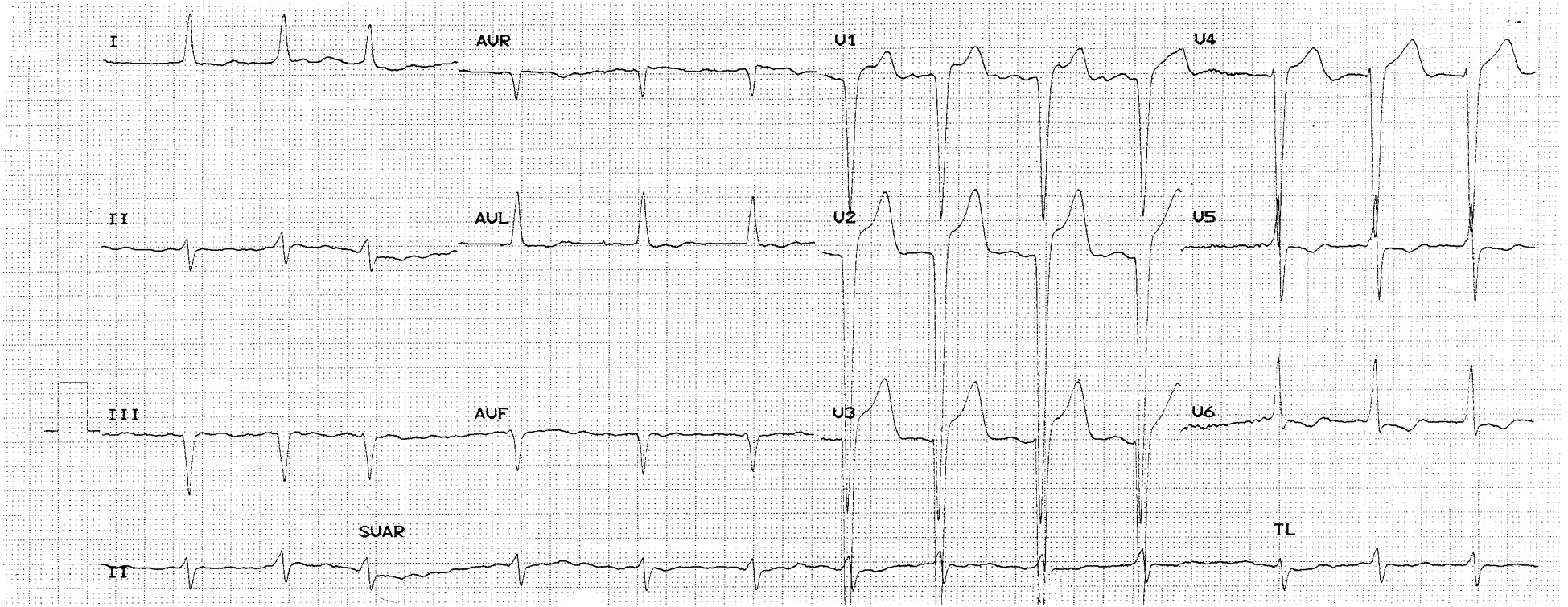


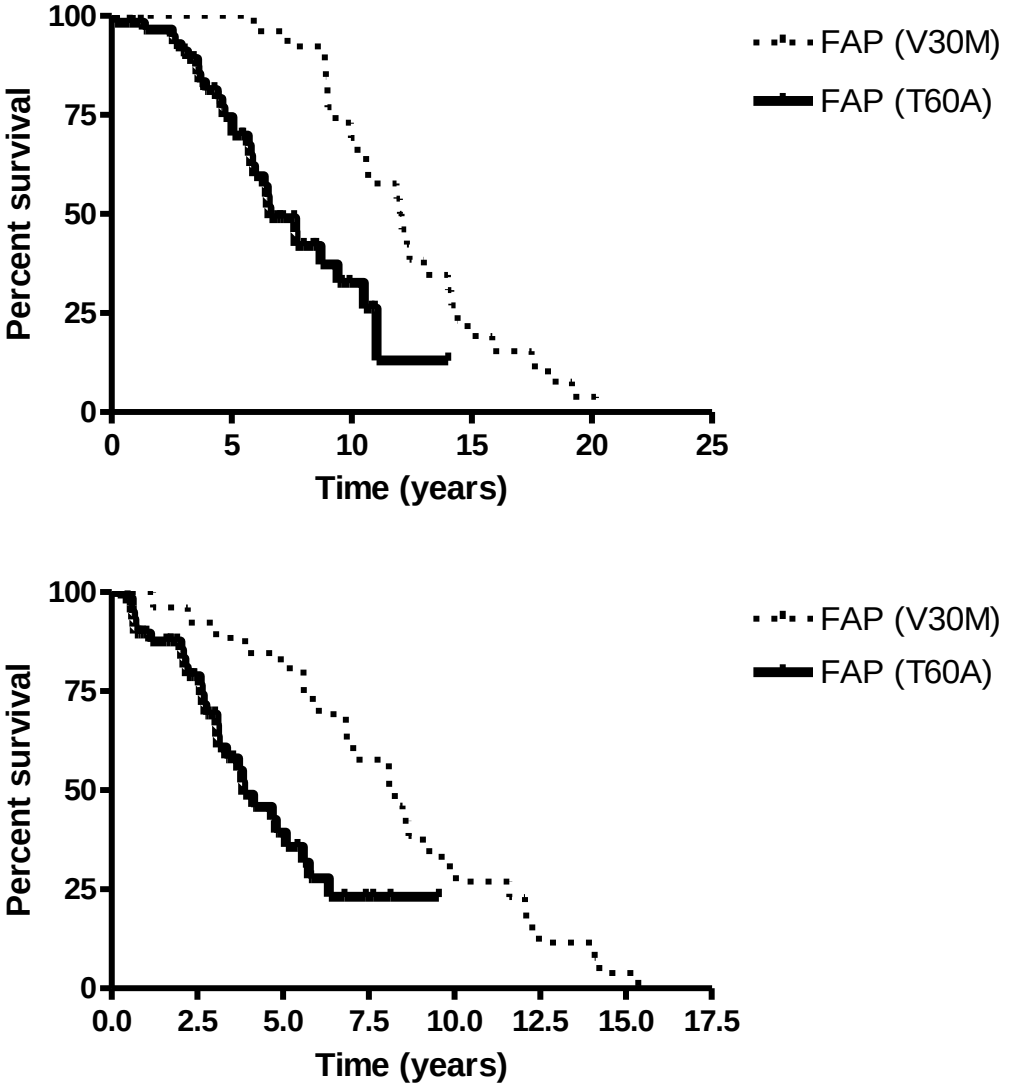
Table 3.2 Relationship between NT-proBNP and echocardiographic parameters.

Characteristic	Unadjusted analysis Regression coefficient (95% CI)	P-value	Multivariable analysis Regression coefficient (95% CI)	P-value
Diastolic dysfunction: for each increase in grade	-16.18 (-40.01, 7.66)	0.18	-	-
LVPWd thickness: for each mm increase	79.62 (45.70, 113.54)	<0.0001	-	-
IVSd thickness: for each mm increase	80.61 (47.07, 114.15)	<0.0001	50.71 (14.94, 86.46)	0.007
Left atrial diameter: for each mm increase	51.72 (22.90, 80.53)	0.001	24.30 (-2.80, 51.40)	0.08
Left atrial area: for each cm^2 increase	46.96 (21.16, 72.77)	0.001	-	-
Ejection fraction: for each unit increase	-25.11 (-35.89, -14.33)	<0.0001	-18.46 (-29.06, -7.86)	0.001

Table 3.3 Neurological features at diagnosis of familial amyloid polyneuropathy associated with TTR T60A variant.
(Patient with multiple sclerosis excluded from analysis)

Autonomic neuropathy	44/59 (75%)
Postural hypotension	28/59 (47%)
Altered bowel habit	
Constipation	8/59 (14%)
Diarrhoea	10/59 (17%)
Alternating diarrhoea and constipation	26/59 (44%)
Upper gastrointestinal tract symptom (early satiety, dyspepsia, dysphagia, vomiting)	15/59 (25%)
Urinary retention	7/59 (12%)
Impotence	26/43 (61%)
Peripheral neuropathy	32/59 (54%)
No peripheral or autonomic neuropathy	12/59 (20%)

Figure 3.4 Kaplan-Meier survival from symptom onset (top) and from diagnosis of amyloidosis (bottom) for 52 non-transplanted FAP T60A patients (thick line) compared to 26 non-transplanted Swedish FAP V30M patients (thin line) (top: p=0.002; bottom: p<0.001).



significantly different between 52 non-transplanted FAP T60A patients and those who underwent OLT (HR for transplanted patients 0.48 (95% CI: 0.16-1.43); p=0.19).

In an analysis of all 60 FAP T60A patients in this series, age (Hazard ratio (HR): 2.49 (95% CI: 1.28-4.85) for each 10 years older; p=0.007), IVS thickness (HR: 0.31 (95% CI: 0.14-0.72) for <17 mm versus ≥17 mm; p=0.006), NT-proBNP (HR: 0.39 (95% CI: 0.16-0.96) for <400 pmol/l versus ≥400 pmol/L; p=0.04), diastolic dysfunction (HR: 0.33 (95% CI: 0.12-0.91) for grade 0-1 versus grade 2-4; p=0.03), LVPW diameter (HR: 0.42 (95% CI: 0.18-0.95) for <17 mm versus ≥17 mm; p=0.04) and weight loss at diagnosis (HR: 2.85 (95% CI: 1.08-7.54) for weight loss versus no weight loss; p=0.03) were associated with reduced survival in univariable analyses. Factors that were significantly associated with reduced survival in multivariable analysis included NT-proBNP (HR: 0.17 (95% CI: 0.03-0.92) for <400 pmol/L versus ≥400 pmol/L; p=0.04) and LVPW thickness (HR: 0.17 (95% CI: 0.03-0.97) for <17 mm versus ≥17 mm; p=0.05). LA area was also a significant predictor of death after adjustment (HR: 9.24 (95% CI: 1.27-67.40) for >20 cm^2 versus ≤20 cm^2; p=0.03). Ten deaths were directly related to cardiac amyloidosis, 5 were sepsis-related, and the precise cause was not ascertained in 14 cases.

Cardiac arrhythmias were documented in 21/60 (35%) patients during the course of their disease including atrial fibrillation in 12 cases, complete heart block in 5 cases and ventricular tachycardia in 3 cases. Seventeen (28%) patients required cardiac device insertion for an arrhythmia (16 pacemakers and 1 implantable cardioverter-defibrillator), 12 of which were inserted prior to establishing the diagnosis of FAP. Six further patients received prophylactic pacemaker insertion during evaluation for potential OLT.

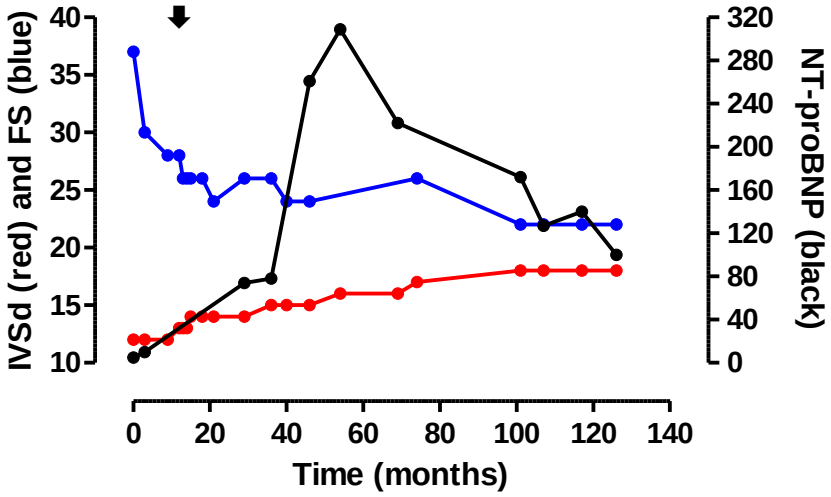
OLT to eliminate hepatic production of variant TTR was performed in 8 patients of median (range) age of 60 (49-65) years. One of these patients, who had severe

cardiac amyloidosis, received a combined cardiac and liver transplant aged 52 years. Median (range) time from onset of symptoms to transplantation was 28.5 (3.0-65.0) months, and 13.5 (2.0-56.0) months from diagnosis of FAP. Standard anti-rejection immunosuppressive regimens were used in accordance with local protocols. Median (range) follow-up from OLT was 4.5 (0.2-8.7) years during which there were no graft failures. Four of 8 OLT patients died, 3 from cardiac amyloidosis and 1 from sepsis, 0.2, 1.0, 1.9 and 3.9 years after surgery. The 4 OLT recipients who died had reduced mobility because of peripheral neuropathy, symptomatic postural hypotension and marked weight loss indicating advanced disease at the time of transplantation in contrast to the 4 patients who survived for more than 5 years among whom these features were not present. There had been progressive neuropathy and cardiac amyloidosis after OLT, the latter of which is highlighted in one patient in Figure 3.5, in all survivors of more than 5 years except the one patient who also received a heart transplant, who remained stable and well at census 100 months after the combined transplant. Median (range) increase in NT-proBNP concentration per month after OLT alone was 54 (1.3-79.8) pmol/L and median (range) increase in IVS wall thickness per year after OLT alone was 1.56 (0.72-2.6) mm.

Discussion

Several types of systemic amyloidosis may involve the heart and given the different prognosis and treatments of the different types,[52] definitive diagnosis with immunohistochemical typing of amyloid, genetic studies and evaluation for a plasma cell dyscrasia is critical. The heart is the chief site of amyloid deposition in senile systemic amyloidosis derived from wild-type TTR, and is very commonly involved in AL amyloidosis, which is by far the most commonly diagnosed type. There is particular scope for misdiagnosis of FAP as AL amyloidosis, since there is frequently

Figure 3.5 Progressive cardiac amyloidosis after orthotopic liver transplantation. Liver transplantation, timing of which is shown by the vertical arrow, was followed by an ongoing increase in inter-ventricular wall thickness (red line), a progressive fall in fractional shortening (blue line) and a marked rise in NT-proBNP (black line).



no obvious family history with the former and the very heterogeneous presentation of the latter often includes autonomic and/or peripheral neuropathy.[67] Chemotherapy, the treatment for AL amyloidosis, has no role in TTR amyloidosis and can be extremely harmful; the presence of an incidental plasma cell dyscrasia in 8% of the current cohort, which is a prevalence comparable with that in the general ageing population, underpinned misdiagnosis and administration of inappropriate chemotherapy in some cases.

The characteristic echocardiographic features of cardiac amyloidosis in the FAP T60A cohort, which are common to all types of amyloid cardiomyopathy, were left ventricular wall thickening and diastolic dysfunction. Systolic function was usually well preserved. Differentiation between TTR and AL amyloid cardiomyopathy is not possible by echocardiography alone,[17] although prognosis of AL amyloid cardiomyopathy is substantially worse than TTR-type in relation to the degree of LV wall thickening.[52] The reasons are poorly understood but the slower accumulation of amyloid in a TTR-type cardiomyopathy[17] and possible toxicity of certain amyloidogenic light chains on myocyte signalling have been postulated as causes.[137] Electrocardiographic analysis revealed attenuated QRS voltages in only 16% of cases in the current series, compared to 45-60% patients with AL cardiomyopathy.[138,139]

The cardiac biomarker NT-proBNP has been shown to be a sensitive indicator of cardiac involvement and prognosis in AL amyloidosis,[31,140] but it has been little studied in TTR amyloidosis. In the present study, there were highly significant relationships between NT-proBNP concentration at diagnosis and both ventricular septal thickness and ejection fraction, consistent with observations of BNP in a small Swedish FAP V30M study.[141] It is noteworthy that NT-proBNP concentration was not associated with degree of diastolic dysfunction. These findings are analogous to AL amyloidosis

in which there is a correlation between fall in NT-proBNP and improvement in systolic function after chemotherapy.[142]

To date, the greatest wealth of published information has been with regard to FAP associated with TTR V30M in which neuropathic features dominate and in which most of the outcome data following liver transplantation exists.[86] This study highlights several important differences between FAP V30M, which is the commonest form of FAP worldwide and FAP T60A, which is the commonest variant in the British population and which constitutes 20% of the FAP patients in the NAC database. The presence of cardiac amyloid in FAP T60A accounts for several of these differences. First, FAP T60A is predominantly a disease of the heart and autonomic nerves with less than one quarter of patients presenting with peripheral neuropathy and there being minimal progression of peripheral polyneuropathy during follow-up. In fact, all of the patients in the current series developed cardiac amyloid during follow-up. In contrast, FAP V30M typically manifests as a progressive autonomic and peripheral sensorimotor polyneuropathy and rarely causes heart failure.[52] Second, FAP T60A characteristically presents at a later age than FAP V30M; the median age at onset of symptoms was 63 years, and only 1 patient presented before age 50 years compared with FAP V30M in which onset during the third to fourth decade of life is most common.[50,143] Third, prognosis in FAP T60A is distinctly worse than in FAP V30M, as is outlined in Figure 3.4, and no doubt in part reflects a later age of onset and the frequency and severity of the cardiac involvement in FAP T60A. The impact of cardiac amyloid in FAP T60A is further emphasised by the fact that all of the independent predictors of survival in this cohort were cardiac parameters (LVPW diameter and LA area by echocardiography, and NT-proBNP concentration), analogous to the impact of cardiac amyloid on survival in AL amyloidosis.[28]

Although OLT has been hailed as an appropriate treatment for FAP, only 7 patients in the cohort underwent OLT and one additional patient, who had advanced cardiac amyloidosis at presentation, received a combined cardiac and liver transplant. The possibility of OLT was ruled out in the remaining patients on grounds of age, advanced disease and patient choice. The potential impact of cardiac amyloid on OLT in FAP T60A patients is highlighted in this series. Firstly, there was evidence of progression of amyloidosis in the hearts of all solitary OLT recipients who survived for more than 5 years. Secondly, overall survival was poor with 4 patient deaths occurring within 5 years from OLT and three of these deaths were related to cardiac amyloid. These observations substantiate the critical significance of cardiac involvement in FAP patients who undergo OLT, both in terms of fitness to undergo major surgery such as OLT and with respect to the course of the amyloid heart disease following OLT. Whilst it remains possible that the rate of disease progression might have been modified following OLT, the overall impression falls in marked contrast with published outcome data following OLT for FAP V30M in which progression of amyloid neuropathy is successfully halted by OLT, especially when OLT is undertaken early in the course of the disease.[86] The progression of cardiac amyloidosis after OLT observed here extends previous reports of 'paradoxical' worsening of amyloid in the heart.[87,144] This cardiac phenomenon has been confirmed by pathologic studies that have revealed a disproportionate excess of wild-type TTR amyloid in the hearts of patients who had undergone OLT as compared to those who had not.[88] The particular propensity for TTR protein to undergo conversion into amyloid fibrils in the heart is compellingly demonstrated by the syndrome of senile systemic amyloidosis in which wild-type TTR is deposited almost exclusively as cardiac amyloid in up to 25% of elderly individuals.[145] The role of OLT for FAP T60A and indeed all "non-V30M" forms of FAP in which cardiac involvement is characteristic, therefore remains most uncertain.

Conclusion

FAP T60A is a progressive condition dominated by cardiac amyloidosis and associated with varying degrees of autonomic and peripheral polyneuropathy. The poor outcome is largely attributable to the presence of cardiac amyloid. The role of OLT in FAP T60A remains uncertain, fuelling much hope for the various novel pharmacologic treatments currently in development, which include drugs to stabilise TTR protein in its non-amyloid conformation, RNA therapeutics to inhibit production of TTR, and monoclonal antibody therapy to eliminate existing amyloid deposits.[100,108]

Chapter 4

Hereditary lysozyme amyloidosis - Phenotypic heterogeneity and the role of solid organ transplantation

Introduction

Hereditary non-neuropathic systemic amyloidosis was first described in 1932 by Ostertag.[56] A number of mutations in genes encoding amyloidogenic variant precursor proteins have been identified: apolipoprotein AI (16 mutations), apolipoprotein AII (4 mutations), fibrinogen Aα-chain (9 mutations) and lysozyme (6 mutations). The phenotype of these conditions is variable in terms of penetrance, age of onset, organ involvement, rate of progression and prognosis, not only within a given amyloidosis type but even between those with the same mutation.

ALys was first described in 1993 by Pepys et al. and in vitro studies with lysozyme have provided seminal insights into amyloid fibrillogenesis.[146] The phenotype of ALys is heterogeneous and includes gastrointestinal symptoms, hepatic rupture, sicca syndrome, petechiae and purpura, renal failure and lymphadenopathy. Lysozyme is a bacteriolytic enzyme that is synthesised in the gastrointestinal tract, in macrophages and in hepatocytes. Six different mutations of the lysozyme gene have been reported to encode amyloidogenic variants of the protein[146-150] and a non-amyloidogenic polymorphism has also been reported.[151]

Aims

The phenotype of ALys has largely been reported in case reports or kindred analyses. ALys tends to be referred to under the umbrella of hereditary non-neuropathic systemic amyloidosis and hence is often labelled as a disease of the kidneys. In this series, which is the largest reported to date and which includes prolonged patient follow-up, there is characterisation of the clinical presentation of this disease with a focus on the dominant non-renal aspects, in particular the gastroenterological and hepatic features. There is also review of its natural history, including the role of solid organ transplantation (liver and kidney) in the face of organ failure.

Methods

Sixteen patients with ALys who were followed up prospectively at the National Amyloidosis Centre (NAC) between 1988 and 2010 were identified retrospectively from the centre database. Diagnosis of ALys required identification of amyloid deposits within tissues (by histology with immunohistochemistry and ^{123}I-SAP scintigraphy in 13 cases and by ^{123}I-SAP scintigraphy alone in 3 cases) in combination with presence of a mutation in the lysozyme gene, corroborated by a family history of amyloidosis and absence of raised inflammatory markers or a clonal disorder. Patients attended the NAC for their initial diagnostic evaluation and were followed up at regular (6 monthly to yearly) intervals for evaluation of organ function including detailed echocardiographic evaluation of the heart and monitoring of whole body amyloid load by serial ^{123}I-SAP scintigraphy. Additional investigations were undertaken when clinically indicated. Amongst cases where organ failure supervened, listing and procedure for organ transplantation was according to local transplant centre protocols.

Results

Patients

Sixteen patients (5 male) with ALys were identified and all were Caucasian. Median (range) age of development of amyloid-related symptoms was 25 (9-70) years, delay from symptom onset to diagnosis of amyloidosis was 2.3 (0-29.3) years, and median (range) age of presentation to the NAC was 45 (18-72) years. Patient 8 was referred for genetic screening and was the only asymptomatic case in the series and remained so until her death aged 72 years. Her sister (Patient 7, Table 4.1) presented with renal amyloidosis aged 66 years.

The clinical heterogeneity of ALys is highlighted in Table 4.1. Presentation was most commonly from sicca syndrome from presumed amyloidotic salivary gland infiltration (6 cases), or with renal dysfunction (5 cases). Presentation with rupture or haematoma of the liver occurred in 2 cases and gastrointestinal (GI) disturbance was the presenting feature in 2 cases. One case was asymptomatic and was detected by family screening. No patient had symptoms or investigations to suggest involvement of the heart by amyloid.

Histology

Amyloid was confirmed by histology in 13 patients from biopsies of the GI tract (7 cases), kidneys (3 cases), explanted liver after rupture (2 cases) and spleen after rupture (1 case). Positive immunohistochemical staining with antibodies against lysozyme and negative staining with all other antibodies confirmed the amyloid fibril protein to be lysozyme.

Serum amyloid P component (SAP) scintigraphy

By the time of diagnosis, all symptomatic patients had amyloid in the liver and spleen and a large total body amyloid load by SAP scintigraphy. The kidneys were obscured by hepatosplenomegaly on the planar images in 5 cases. Amongst the remaining cases, the kidneys exhibited abnormal uptake in each of 6 with renal dysfunction and 2 of 5 without renal dysfunction. The asymptomatic patient (Patient 8, Table 4.1) had unequivocal amyloid in the spleen and kidneys by SAP scintigraphy but none in the liver. This patient did not have any tissue biopsies. Interestingly, amyloid did not continue to accumulate within native organs despite more than eight years follow-up in 4 patients, contrasting recurrence of amyloid within 3 transplanted livers over a shorter time period.

DNA Analysis and Genetics

Twelve patients were heterozygous for a mutation in the lysozyme gene which encodes the D67H variant, 2 cases carried the W64R variant and 1 case each carried the I56T and D67G variants, all of which have been previously reported. Four kindreds accounted for 11 of 12 patients with the D67H variant (Table 4.1).

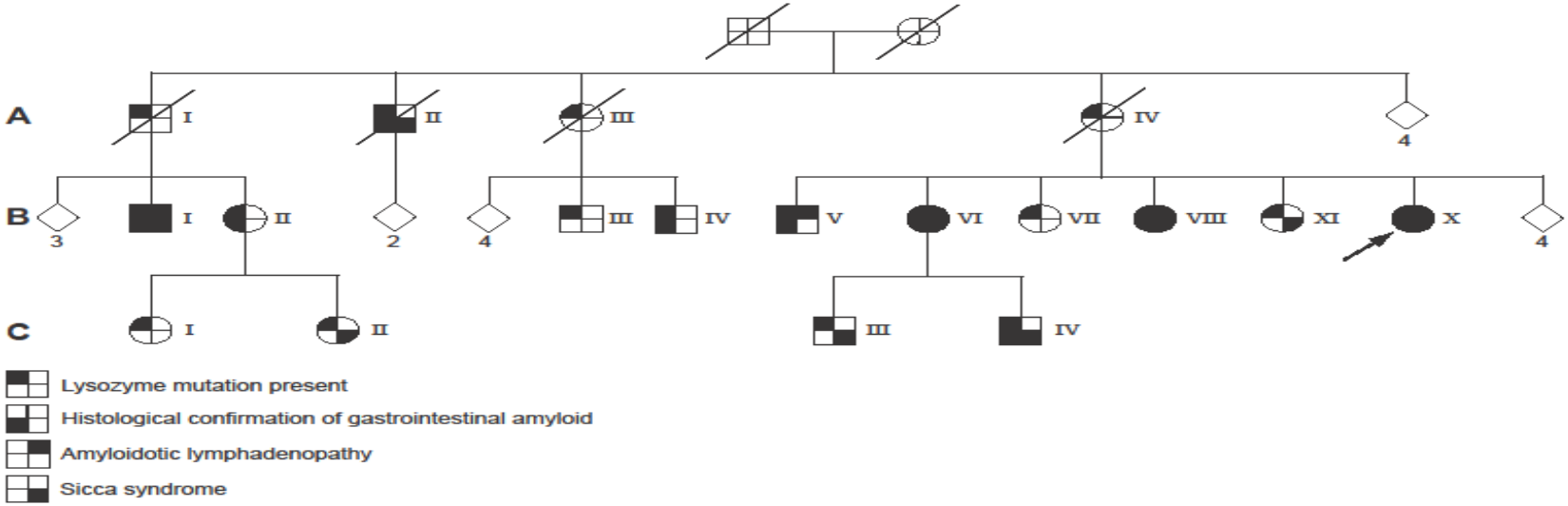
A family history of ALys was evident in every patient in the cohort. All except Patient 6 had evidence of a 1st and 2nd degree relative with biopsy-proven amyloid or characteristic features of ALys. Patient 6 had a 2nd degree relative with amyloid and is a 4th degree relative of Patients 4 and 5. The highly penetrant nature of ALys is highlighted in the family tree of patient 14 (Figure 4.1). The proband from this family developed sicca syndrome aged 13 years. She had recurrent friable lesions on oesophagogastroduodenoscopy, which presented as abdominal pain and haemorrhage and, aged 30 years, underwent an emergency small intestinal resection for spontaneous small intestinal perforation. Two of the proband's sisters (Figure 4.1, BVI and BVIII)

Table 4.1 Clinical characteristics of ALys patients.

Patient number (Gender)	Family Number	Lysozyme variant	Mode (age) of presentation	Age at diagnosis (Organ biopsy)	Additional clinical features	Gastrointestinal symptoms/Upper gastrointestinal tract endoscopic findings	Dead (D) or Alive (A)/ Time from diagnosis to death or census (years)
1 (F)	1	D67H	Sicca syndrome (11)	28 (GI)	B	Nausea and vomiting / Oesophageal and gastric nodules	A / 2.5
2 (M)	1	D67H	Sicca syndrome (25)	54 (GI)	-	Dyspepsia / Extensive upper gastrointestinal tract ulceration	A / 7.6
3 (F)	1	D67H	Sicca syndrome (11)	27 (GI)	-	Dyspepsia / Gastritis	A / 21.6
4 (F)	2	D67H	Liver rupture (24)	24 (Liver)	S	Haemorrhage / Haemorrhagic gastritis and ulceration	A / 5.8
5 (M)	2	D67H	GI haemorrhage (9)	9 (GI)	L, LN	Haemorrhage / Multiple bleeding polyps from oesophagus to duodenum, haemorrhagic gastritis	D / 17.9
6 (M)	2	D67H	Liver haematoma (34)	47 (GI)	R, B	Haemorrhage / Peptic ulcer, friable lesions	A / 15.5
7 (F)	3	D67H	Renal dysfunction (67)	67 (Kidney)	-		D / 4.8
8 (F)	3	D67H	None/FHx	72 (-)	-		D / 2.0
9 (M)	4	D67H	Sicca syndrome (20)	22 (Kidney)	R, S	Dyspepsia and diarrhoea / Normal	A / 29.6
10 (F)	4	D67H	Renal dysfunction (51)	51 (Kidney)	-	Anaemia / -	A / 12.1
11 (F)	4	D67H	Renal dysfunction (51)	51 (-)	S	Haemorrhage, diarrhoea / -	D / 10.9
12 (F)	5	D67H	Sicca syndrome (13)	34 (Liver)	L, S, B	Abdominal pain / Peptic ulcer, friable lesions	A / 9.1
13 (F)	6	I56T	Renal dysfunction (70)	71 (-)	B		D / 5.8
14 (F)	7	W64R	Sicca syndrome (13)	34 (GI)	S, LN	Haemorrhage / Peptic ulcer, friable lesions	A / 6.6
15 (F)	7	W64R	GI haemorrhage (52)	52 (GI)	S	Haemorrhage / Peptic ulcer, friable lesions	A / 10.5
16 (M)	8	D67G	Renal dysfunction (29)	32 (Spleen)	S, B, SP		A / 1.8

S= Sicca syndrome, B= Bruising, FHx = Family history, LN=Lymphadenopathy, SP=Splenic rupture, R=Renal dysfunction, L=Liver rupture

Figure 4.1 Family tree of patient 14. The proband is indicated by the arrow. Circles represent females and squares represent males. The diamonds with boxes below them represent the number (as stated in the box) of unaffected siblings who are either asymptomatic or negative for the ALys mutation. Dead individuals are indicated by a diagonal line through the symbol.



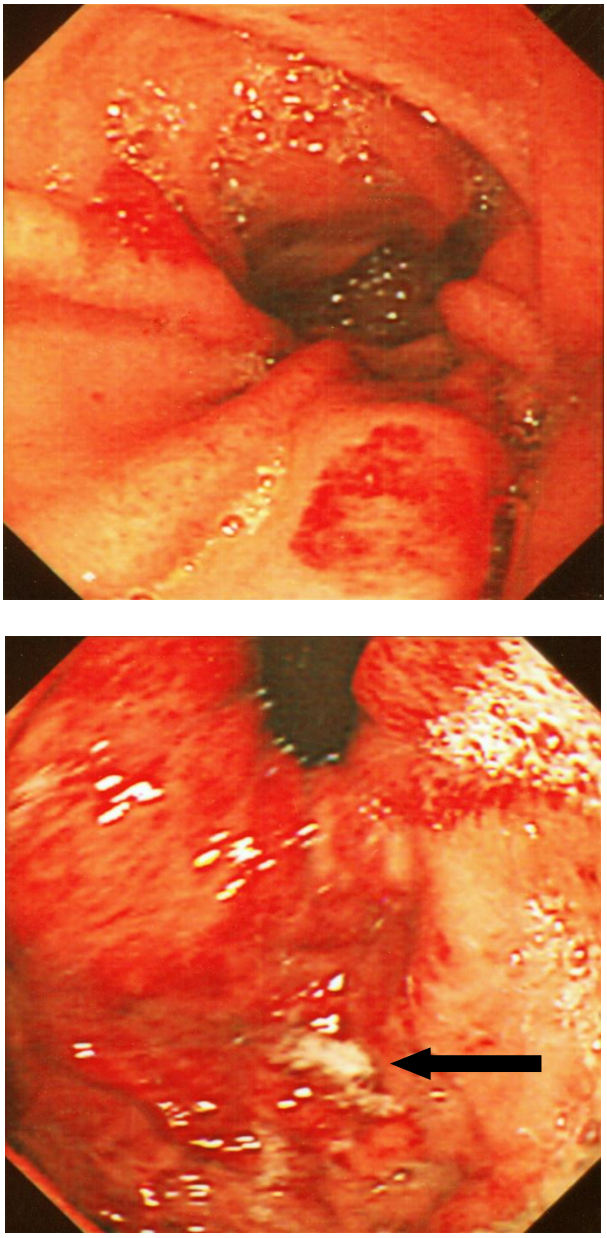
also had endoscopically confirmed friable lesions which presented with early satiety and haematemesis, respectively. The proband's nephew (Figure 4.1, CIV), developed abdominal pain secondary to peptic ulceration at the age of 14 years. Two first cousins of the proband, (Figure 4.1, BIV and BII), developed anaemia on the background of overt gastrointestinal haemorrhage in their third decade which was associated respectively with oesophageal haemorrhagic nodules and gastric friable lesions.

Clinical Course and Outcome

Endoscopic investigation during the course of the illness was prompted by GI haemorrhage in 6 (38%) cases (Figure 4.2), by dyspepsia in 2 cases and by nausea and vomiting in 1 case. Nine of 10 patients who underwent oesophagogastroduodenoscopy had endoscopic abnormalities such as peptic ulceration (4 cases), oesophageal or gastric nodules and haemorrhagic gastritis. Contact bleeding was observed on several endoscopic evaluations, even at points where the mucosa looked macroscopically normal and in the absence of clotting or platelet abnormalities. The 4 patients with peptic ulceration all received proton pump inhibitor (PPI) therapy although whether this was clinically beneficial is not clear; at least one patient had further GI bleeding whilst on chronic PPI therapy. Several patients (Table 4.1, Patients 6, 9 and 11) had diarrhoea associated with weight loss and another (Patient 14) had a spontaneous small intestinal perforation which required resection and subsequent intestinal anastomosis, suggesting fragility of the intestinal wall from amyloid infiltration. Patient 6 had steatorrhoea with a faecal fat concentration of 60 mmol/l (normal <18 mmol/l) and subsequently presented with intestinal perforation secondary to a combination of diverticular disease and extensive GI amyloid infiltration, for which he required a Hartmann's procedure.

Despite extensive hepatic infiltration by amyloid by the time of diagnosis, liver function tests remained relatively preserved with a normal bilirubin in all patients, and a

Figure 4.2 Oesophagogastroduodenoscopic views of the stomach of Patient 4 with ALys after presentation with upper gastrointestinal tract haemorrhage : antral gastritis (top) and haemorrhagic gastritis of the fundus with ulceration (arrow).



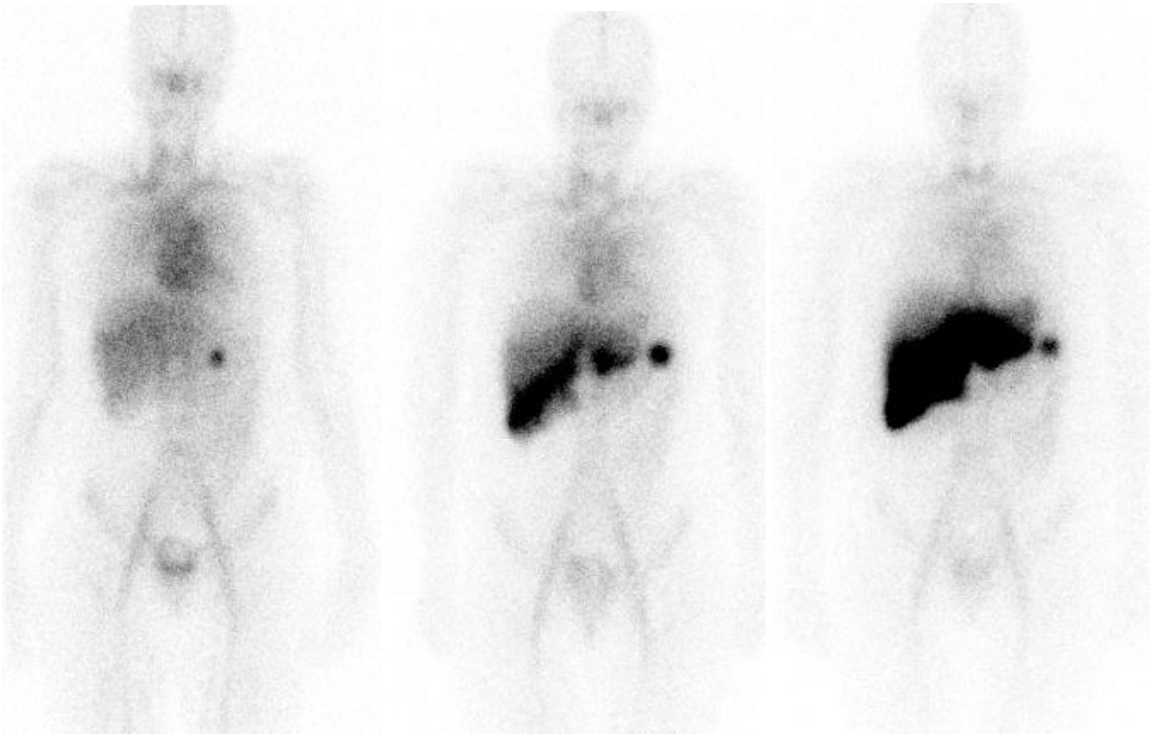
mildly elevated alkaline phosphatase (median (range) 77 (62-295) U/L) and aspartate aminotransferase values in only 2 and 4 cases respectively. Median (range) serum albumin at diagnosis was 43 (27-50) g/L. Three patients (Table 4.1, Patients 4, 5 and 12) spontaneously ruptured their livers aged 24, 15 and 34 years respectively; each underwent emergency orthotopic liver transplantation (OLT) accompanied by perioperative splenectomy because of splenic fragility. The remaining OLT recipient (Patient 15) underwent the transplant because she had heavy hepatic amyloid infiltration and a strong family history (mother and sister) of spontaneous liver rupture. Patients 4 and 5 were siblings and there had been a history of spontaneous hepatic rupture in both their father and paternal grandfather. The mother of Patient 12 also had a spontaneous hepatic rupture.

At census, there were no hepatic graft failures 1.7, 5.8, 9.0 and 11.0 years from OLT. Serial SAP scintigraphy however, showed asymptomatic amyloid recurrence within ~3 years in 2 patients (Table 4.1; Patients 4 and 5; Figure 4.3) and amyloid was present in the graft of a third OLT recipient (Patient 12) by the time she first presented to the NAC 7.8 years after the procedure. There was no evidence of amyloid recurrence in the remaining patient at census 1.7 years after OLT.

Ten cases presented with or developed sicca syndrome during follow-up, due to presumed salivary gland infiltration by amyloid. Sicca syndrome frequently pre-dated the diagnosis of amyloidosis by several (range 3-30) years. Other forms of ‘soft tissue’ amyloid infiltration manifested as petechiae in 5 patients and 2 patients (Patients 5 and 14) had evidence of amyloidotic lymphadenopathy; the latter was widespread in Patient 14 and was similarly so in 3 affected siblings and a third degree relative (Figure 4.1).

Amyloidotic renal dysfunction occurred in 7 patients during the course of their illness and was progressive, culminating in end-stage renal disease (ESRD) in 5 of these cases (Table 4.1; Patients 7, 9, 10, 11 and 16). Median (range) 24 hr urinary protein

Figure 4.3 Serial anterior whole body ^{123}I-SAP scintigraphy of Patient 4 after OLT - recurrence and progression of lysozyme amyloid in the liver graft after OLT for hepatic rupture and progression of amyloid in a splenunculus 5 (left), 41 (middle) and 65 (right) months after OLT.



loss among those presenting with renal dysfunction was only 0.3 g (0-4.0 g); a single patient had nephrotic-range proteinuria but was not clinically nephrotic. There was no evidence of renal dysfunction in the remaining 9 patients during follow-up. Median time to end-stage renal failure from discovery of renal dysfunction by Kaplan-Meier analysis was 11.0 years.

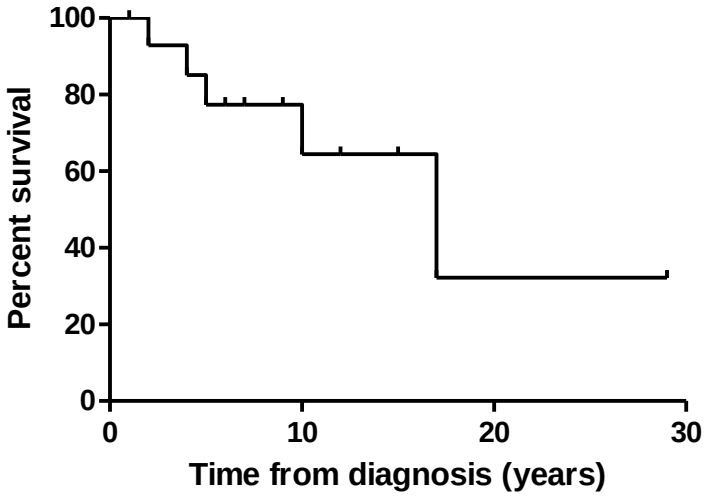
Living donor renal transplantation (RTx) was undertaken in 3 patients (Table 4.1; Patients 9, 10 and 16), pre-emptively in 2 cases and 0.2 years after ESRD in Patient 10. All 3 patients were alive at census 6.6, 0.8 and 1.8 years after RTx respectively, without evidence in any graft of amyloid recurrence. RTx is also known to have taken place in 2 relatives with ALys of patients in this cohort without evidence of graft failure after 8 years (brother of Patient 10) and 8 months (niece of Patient 14) from RTx.

The median estimated survival from diagnosis of amyloidosis in this series was 17.9 years (Figure 4.4) and despite median follow-up from symptom onset of 24.7 years only 4 of 15 patients died (one patient remained asymptomatic throughout her disease course and was therefore excluded from the latter analysis).

Discussion

The hereditary non-neuropathic systemic amyloidosis syndromes are characterised by an autosomal dominant mode of inheritance and are typically associated with variable penetrance. Hereditary fibrinogen A-α chain and apolipoprotein AII amyloidosis cause renal failure, apolipoprotein AI amyloidosis is associated with renal, hepatic and cardiac disease and gelsolin amyloidosis with corneal lattice dystrophy, cranial neuropathy and occasionally, renal failure.[57] ALys is the most clinically heterogeneous condition in this group and may mimic the more common AL amyloidosis.[72] ALys is very penetrant

Figure 4.4 Kaplan-Meier survival from diagnosis of amyloidosis in ALys patients.



however, and a family history of amyloidosis is usually present in marked contrast to other forms of hereditary systemic amyloidosis.[67,152] Distinguishing between ALys and AL amyloidosis is critical for correct patient management. The median survival of nearly 18 years from diagnosis in ALys contrasts systemic AL amyloidosis in which the median survival with modern chemotherapy regimens is ~3-4 years from diagnosis. In fact, median survival in the absence of chemotherapy among patients with AL amyloidosis is ~1 year, whereas there is no role for chemotherapy in ALys. One patient in this series (Table 4.1; Patient 7) received inappropriate chemotherapy for presumed AL amyloidosis prior to review in the NAC. It is imperative therefore, that patients with systemic amyloidosis are screened for a family history of similar disease and undergo genetic sequencing, particularly if the amyloid fibril protein remains uncertain despite immunohistochemical staining of amyloidotic tissue, which may be the case in up to 40% of patients with AL amyloidosis.[67]

Symptomatic gastrointestinal amyloid is relatively unusual in the other forms of hereditary non-neuropathic systemic amyloidosis but appears prevalent in this ALys cohort. Gastrointestinal haemorrhage associated with endoscopically visible, friable lesions was common in this series, and has previously been reported in a French family with ALys.[153] It would seem sensible therefore to give consideration to lifelong prophylactic proton pump inhibitor use in patients with ALys who have GI amyloid to try and reduce risk of upper GI tract haemorrhage. Whether this practice is clinically beneficial however, cannot be concluded from this case series. Three patients in this series had severe diarrhoea and weight loss including one with fat malabsorption suggestive of small intestinal amyloid, which has also been reported previously in ALys.[154]

Hepatic amyloid, detected by SAP scintigraphy, was virtually universal at diagnosis in these patients, along with a large total body amyloid burden. Interestingly,

liver function tests were barely deranged and remained relatively stable during follow-up, which contrasts AL amyloidosis in which patients with similar SAP scintigraphic appearances have markedly deranged liver biochemistry and may develop hepatic decompensation.[155] Both this and the finding that median time from presentation with renal dysfunction to ESRD in this cohort was 11.0 years indicate that the natural history of ALys is markedly slower than AL and other types of hereditary amyloidosis.[152,156] The mechanism by which amyloid accumulation in native organs appears to abate in ALys, once patients have accumulated a large total body amyloid burden, remains unclear.

Hepatic haemorrhage and/or rupture occurred in 3 of 12 (25%) patients with ALys associated with the D67H variant in this series. Since the diagnosis of amyloid was only established after liver rupture in all 3 of these cases, it may be that the prevalence suggested here is falsely high. Patient 5's explanted liver was noted to be fragile and friable with loss of the reticulin framework[157] and that of Patient 4 was 3 kg, which is approximately twice the weight of a normal liver.[158] Although the extent of amyloid infiltration may contribute to risk of haemorrhage, it is notable that liver rupture in AL amyloidosis, which can also be associated with massive hepatomegaly is exceptionally rare. Emergency OLT among these 3 patients was life-saving and appears to have a good outcome in terms of graft survival, despite evidence of amyloid recurrence in the graft, with 100% 1- and 5-year graft survival in this small series. One of the cohort had a "prophylactic" OLT on a background of extensive hepatic amyloidosis and a family history of hepatic rupture. In a climate of donor shortages, the merits of such an approach remain uncertain. It would however seem reasonable to recommend a pre-emptive OLT assessment in those with confirmed ALys, particularly if there is a family history of hepatic rupture or hepatic haematoma.

Soft tissue amyloidosis, one manifestation of which is sicca syndrome, is recognised in both ALys and AL amyloidosis.[33,159] Sicca syndrome frequently predated vital organ dysfunction in this cohort and should prompt a search for amyloid deposits in any patient with a family history of ALys. Indeed, 2 patients in this series were diagnosed in precisely this fashion. Artificial saliva or tears may provide symptomatic relief. Other 'soft tissue' manifestations of amyloid in this cohort were bruising and lymph node enlargement, which are usually considered to be almost pathognomonic of AL amyloidosis.[72] Patient 14 had evidence of widespread amyloidotic lymphadenopathy, which has not been reported before in ALys.

Renal dysfunction is common in all forms of hereditary non-neuropathic systemic amyloidosis and 5 of 16 cases in this series reached ESRD. Age at presentation and rate of renal progression varied widely, even among patients from the same family who carry the same mutation, evident from Family 4 in this series and consistent with previous reports.[148,160,161] Despite a large total body amyloid burden and a complete lack of available therapy to halt amyloid accumulation in ALys, outcomes among 3 RTx recipients were excellent with functioning renal allografts and no recurrence of amyloid 0.8, 1.8 and 6.6 years after RTx. To date, there has never been a report of renal allograft failure secondary to recurrent amyloid in ALys despite ongoing production of amyloidogenic precursor protein.[147,161,162]

Conclusion

ALys is a rare form of hereditary non-neuropathic systemic amyloidosis which is clinically heterogeneous and may present with hepatic rupture, gastrointestinal haemorrhage, sicca syndrome, purpura and petechiae, lymphadenopathy or renal dysfunction. There is considerable overlap between the clinical features in ALys and AL amyloidosis and it is important to distinguish between these diagnoses since there is

no role for chemotherapy in ALys. Clinical penetrance in the presence of an amyloidogenic lysozyme mutation is high, evident by the obvious family history in all cases within this cohort. Although there is no amyloid-specific therapy for ALys, which is therefore managed symptomatically, the condition has a slow natural history and amyloid accumulation may abate in some patients. RTx for ESRD and OLT for cases of liver rupture appear to be very successful with excellent medium-term graft function and patient survival.

Chapter 5

A prospective study of nutritional status in AL amyloidosis

Introduction

Weight loss is common amongst patients with AL amyloidosis but is frequently not appreciated.[163] Reduced food intake and/or increased energy expenditure secondary to metabolic derangements may contribute. Studies of weight loss in other diseases, typically cancers, have focused on the complex interplay of cytokines, such as TNF-α, IL-1, IL-6 and IFN-γ,[164] which may exert their effects on the gastrointestinal tract or central nervous system.[165] This metabolic syndrome of cachexia can negatively impact upon quality of life, response to therapy and survival.[166]

Weight loss has been found to be a predictor of poorer survival in patients with AL amyloidosis.[22,167,168] One prospective study of 106 patients with AL amyloidosis highlighted that weight loss was a prominent feature, especially in those with cardiac amyloid and identified several simple anthropometric and biochemical measures of nutritional state that correlated with survival in a heterogeneous population which comprised patients, who had received treatment (the majority) as well as those who were treatment-naive.[163] There has only been one previous published study that has included a formal assessment of nutritional status prior to commencement of chemotherapy and this study looked at its impact on quality of life (QOL) in patients with AL amyloidosis.[116] QOL is a multi-layered subjective experience governed by several parameters.[169] Attempts have been made to give it weight and incorporate it into decision-making when considering treatment regimens for different diseases.[170] Inclusion of QOL during assessment of clinical state is often considered 'holistic' and

since it takes into account physical, psychological and social factors, is considered by some to be essential whenever the benefits of interventions are being determined, especially among individuals with potentially fatal conditions in whom a cure may not be the primary objective. One prospective study determined QOL in patients with AL amyloidosis who were treated with high-dose melphalan and autologous stem cell transplantation and found measurable and sustained improvements in QOL.[171] There have been several studies looking at the relationship of nutritional status and QOL in other patient groups; the elderly,[172] dialysis patients,[173] and patients with various cancers.[174-176]

Methods

Aims

The aim of this study was to determine the disease-specific clinical and biochemical parameters associated with malnutrition in consecutive, newly-diagnosed, treatment-naïve patients with AL amyloidosis and furthermore the effect of malnutrition on QOL and survival. There has been a lot of research into prognostic models of survival in systemic AL amyloidosis, with a focus on the effect of cardiac amyloid on such. Hence there has been widespread interest in staging systems incorporating cardiac parameters, such as the Mayo staging system.[112] Malnourished status potentially encompasses the impact of other affected organs besides the heart and another aim of the study was to assess its strength as a prognostic tool versus the Mayo staging system. There was use of a nutritional assessment tool with a continuous scoring system (Patient-Generated Subjective Global Assessment) for these purposes with the view that if there was a significant influence of malnutrition on QOL and survival then such a tool might be useful to gauge the benefits of a nutritional

intervention on these two important patient outcomes, as has been proven in other conditions in which this tool has been used.[177]

Patients

All eligible patients who were reviewed at the UK National Amyloidosis Centre (NAC) between April and December 2009 were invited to participate in the study. Inclusion criteria were as follows: 18 years and over; newly diagnosed biopsy-proven AL amyloid; no prior chemotherapy but requiring chemotherapy for systemic AL amyloidosis; able to give written informed consent for entry into the study. Exclusion criteria were as follows: localised AL amyloidosis, non-AL amyloidosis or suspected AL amyloidosis.

Each patient was assessed at baseline and at 6 monthly intervals until death or census. Census occurred in September 2011, more than 18 months after enrolment into the study of the last patient. Baseline evaluation included the following: detailed clinical assessment including lying and standing blood pressure and nutritional status; biochemical tests of renal (including proteinuria) and hepatic function, assessment of NT-proBNP and Troponin T concentration, serum free light chain concentration and immunoelectrophoresis of serum and urine, electrocardiography and echocardiography. Organ involvement by amyloid was determined according to the international consensus criteria.[114] and Mayo disease stage was defined according to cardiac biomarkers, as previously described.[112]

Nutritional assessments, including body mass index (BMI) and Patient-Generated Subjective Global Assessment (PG-SGA) were carried out on patients at their baseline visit to the NAC. BMI forms a part of the Malnutrition Universal Screening Tool, which is a tool supported by the Britsh Association of Parenteral Nutrition (http://www.bapen.org.uk) to assess nutritional risk and which also comprises

assessment of weight loss over the prior 3-6 months and whether there has been or will be no oral intake for >5 days. A BMI< 20kg/m^2 is the cut-off for nutritional risk according to this tool and was taken as the cut-off for analysis in this study. QOL was assessed at baseline visit with the EORTC QLQ-C30 questionnaire. An attempt was made to acquire information on other follow-up outcomes besides survival, such as cause of death, via a letter sent to the referring doctor and GP (Appendix 2), but there were few responses and those that were received were often incomplete or lacked sufficient detail.

Statistical analysis

Disease and patient-specific factors associated with the PG-SGA score were identified through linear regression analysis. Cut-points for the factors were chosen by their clinical relevance or in line with previously published data. All factors which were of statistical significance ($p<0.10$) in univariable analyses were included in the multivariable analysis. The assumptions underlying the regression analysis were checked by a study of the residuals and found to be satisfactory. Kaplan-Meier analysis and log-rank tests were used to assess and compare the survival experience between different categories of PG-SGA score of the patients in the cohort. A Cox proportional hazards regression analysis was performed to assess the independent effect on survival of PG-SGA after adjusting for those variables found to be significantly associated with survival in univariable Kaplan-Meier analyses. The Pearson correlation coefficient was determined to assess the linear relationship between the QOL score (both the global score and each of the functional and symptom scale components) and the actual value of PG-SGA. To overcome the problem of multiple testing for these coefficients, a significance level of 0.01 was used.

Results

Patient characteristics

One hundred and ten (66 male) consecutive, newly diagnosed, treatment–naïve patients with systemic AL amyloidosis were enrolled into the study between April and December 2009. Patient characteristics are summarised in Table 5.1. Median (range) age of presentation to the NAC was 66 (42-88). Median (range) time from symptom onset to baseline evaluation was 6 (0-28) months. Eighty-one patients (74%) had a lambda light chain secreting plasma cell dyscrasia and 29 patients (26%) an amyloidogenic kappa-secreting plasma clone. Thirty-five patients (32%) had single organ involvement by amyloid, 46 (42%) had 2 organ involvement and 29 (26%) had 3 or more organs involved according to the international amyloid consensus criteria. The kidneys (85 patients) and heart (69 patients) were the commonest organs to be affected by amyloid. Mayo disease stage at baseline was I, II and III in 16, 35 and 59 patients respectively.

Scored PG-SGA assessment

At baseline evaluation sixty-six patients (60%) stated that they had lost weight over the preceding 6 months, of whom 15 patients had noticed weight loss over the preceding 2 weeks. Food intake, as estimated by the patient, was less than normal over the previous month in 61 (55%) patients, normal in 45 (41%) and increased in 4 (4%) cases. Diet was quantitatively reduced but remained qualitatively unchanged among 51/61 (84%) patients who had eaten less over the preceding month and was both quantitatively and qualitatively altered among the remainder. The main reasons for reduced food intake were reduced appetite (43%), altered taste of food (27%), early satiety (25%) and fatigue (21%) (Table 5.2).

Table 5.1 Patient characteristics at recruitment.

Patient characteristics at baseline	**Value**
Total number (N) of patients	110
Age at recruitment; median years (Inter-quartile range(IQR))	66 (60, 74)
BMI at recruitment; median kg/m^2 (IQR)	25 (23, 29)
Gender; N. (%):	
Male	66 (60.0%)
Female	44 (40.0%)
Plasma cell dyscrasia; N. (%):	
Kappa	29 (26.4%)
Lambda	81 (73.6%)
Amyloid organ involvement (by the international amyloid consensus criteria); N. (%):	
1	35 (31.8%)
2	46 (41.8%)
≥3	29 (26.4%)
Kidney involvement; N. (%):	85 (77.3%)
Cardiac involvement; N. (%):	69 (62.7%)
Liver involvement; N. (%):	16 (14.6%)
Gastrointestinal involvement; N. (%):	5 (4.6%)
Autonomic neuropathy; N. (%):	16 (14.6%)
Peripheral neuropathy; N. (%):	10 (9.1%)
CKD Stage, N. (%)	
≤3	86(78.2%)
>3	24(21.8%)
PG-SGA score; median (IQR)	9.5 (2, 14)
Dialysis; N. (%):	9/85 (10.6%)
Proteinuria; median g/L (IQR)	2.6 (0.6, 6.2)
Albumin (normal ≥35); median g/L (IQR)	33 (25.75, 40)
Bilirubin (normal <19); median µg/L (IQR)	7 (4, 11.5)
Gamma-glutamyl transferase (normal ≤61); median U/L (IQR)	54 (28, 177)
Alkaline phosphatase (normal ≤129); median U/L (IQR)	105 (70, 175)
Mayo Stage; N. (%):	
I	16 (14.5%)
II	35 (31.8%)
III	59 (53.7%)

Table 5.2 Symptoms impeding food intake at recruitment.

Symptoms impeding food intake	Number (Percent) patients
Number of patients	110
No symptoms	47 (42.7%)
Lack of appetite	47 (42.7%)
Food tastes strange	30 (27.3%)
Early satiety	27 (24.6%)
Fatigue	23 (20.9%)
Dry mouth	23 (20.9%)
Problems swallowing	18 (16.4%)
Nausea	13 (11.8%)
Pain	12 (11.0%)
Food smells disagreeable	9 (8.2%)
Constipation	9 (8.2%)
Other factors	9 (8.2%)
Mouth ulcers	8 (7.3%)
Diarrhoea	8 (7.3%)
Vomiting	5 (4.6%)

The PG-SGA score indicated that 14 (13%) patients needed no additional nutritional recommendations (score of 0-1), 24 (22%) cases required nutritional education with pharmacological intervention for management of symptoms (score of 2-3), and 72 (65%) patients required specialist nutritional intervention including 57 (52%) cases who were critically in need of nutritional intervention (score ≥9) (Figure 5.1). Only 4 (<4%) patients had a BMI <20 at baseline, and there was no correlation between BMI and nutritional state, as assessed by PG-SGA (r=-0.14).

Factors significantly associated with a higher PG-SGA score in the multivariable regression analysis are outlined in Table 5.3. An elevated alkaline phosphatase, presence of autonomic neuropathy, a greater number of organs involved by amyloid and a higher Mayo stage were significantly and independently associated with a higher PG-SGA score.

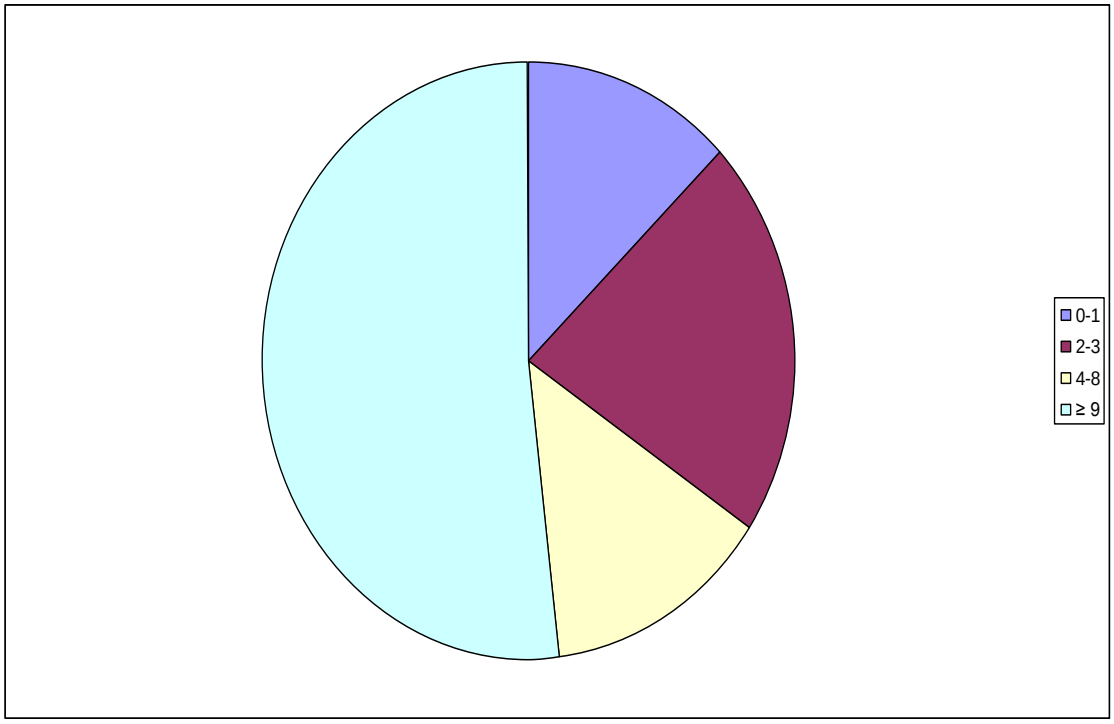
Quality of life

There was a highly significant and consistent relationship between PG-SGA score and QOL measures by the EORTC QLQ-C30 (Table 5.4). Higher PG-SGA scores were associated with poorer overall QOL ($p<0.0001$). There was also a significant association between higher PG-SGA scores and lower scores in all of the functional scales ($p<0.001$) and a significant inverse correlation with all of the symptom scales or items except for dyspnoea ($p\leq0.01$).

Survival

At census with a median (range) follow-up period of 1.2 (0 – 2.4) years, 63 (57%) patients died. PG-SGA score at baseline was the only independent predictor of patient survival [HR: 6.2 (95% CI: 1.2-32.4) for a PG-SGA score 4-8 versus a PG-SGA score 0-1 ($p=0.03$) and HR: 6.4 (95% CI: 1.4-30.2) for a PG-SGA score >8 versus a PG-SGA

Figure 5.1 PG-SGA scores at recruitment.



Nutritional recommendations:

0–1 : does not require nutritional intervention.

2–3 : patients and relatives require nutritional education provided by a specialist in nutrition (or other clinician) with pharmacological intervention based on the symptoms and the patient's data.

4–8 : requires intervention of a specialist in nutrition in conjunction with the attending physician based on the patient's data and symptoms.

≥9 : indicates a critical need to improve the management of the patient's symptoms together with a nutritional intervention.

Table 5.3 Clinical factors associated with PG-SGA score at recruitment.

Characteristic	Unadjusted analysis		Multivariable analysis	
	Regression coefficient (95%CI)	***P*-value**	**Regression coefficient (95%CI)**	***P*-value**
ALP (IU/l) > 129 versus ≤129	 4.69 (2.17, 7.21)	 <0.001	 3.61 (1.30, 5.91)	 0.002
CKD 4-5 versus 1-3	 1.66 (-1.58, 4.85)	 0.30		
PNS	4.64 (0.23, 9.05)	0.04	-0.37 (-4.76, 4.02)	0.87
ANS	7.83 (4.48, 11.18)	<0.001	4.33 (0.55, 8.1)	0.03
N. organs involved 1-2 ≥3	 Ref 7.95 (5.44, 10.46)	 <0.001	 Ref 4.26 (1.07, 7.44)	 0.009
Mayo Stage Stage 1 Stage 2 Stage 3	 Ref 2.84 (-0.93, 6.62) 7.04 (3.52, 10.57)	 0.14 <0.001	 Ref 1.65 (-1.65, 4.95) 3.84 (0.64, 7.03)	 0.32 0.02
Proteinuria (g/24hrs) < 0.5 0.5-3 > 3	 Ref 2.41 (-1.11, 5.93) -2.06 (-5.37, 1.24)	 0.18 0.22		

Table 5.4 Median (IQR) QOL scores according to PG-SGA score at recruitment.

QOL Parameter	Patient Generated Subjective Global Assessment Score				
	0-1	2-3	4-8	≥9	P-value
Functional Scale					
Global QOL	75(54.2-83.3)	62.5(50.7-66.7)	41.7(33.3-66.7)	33.3(16.7-43.8)	<0.001
Physical functioning	89.2(80.0-93.3)	66.7(58.3-81.7)	60(46.7-83.4)	40(26.7-66.7)	<0.001
Role functioning	100(70.9-100)	50(33.3-83.3)	33.3(16.7-66.7)	16.7(0-50)	<0.001
Emotional functioning	91.7(68.8-97.9)	83.3(66.7-91.7)	83.3(54.2-91.7)	66.7(41.7-83.3)	<0.001
Social functioning	100 (66.7-100)	83.3(66.7-100)	66.7(33.3-83.3)	33.3(0-50)	<0.001
Cognitive functioning	100 (83.3-100)	83.3(79.2-100)	100(83.3-100)	66.7(50-100)	<0.001
Symptom scale					
Fatigue	16.65(2.8-30.5)	33.3(22.2-47.2)	44.4(33.3-61.5)	77.8 (66.7-88.9)	<0.001
Appetite loss	0(0-0)	0(0-0)	33.3(0-50)	66.7(33.3-100)	<0.001
Nausea and vomiting	0(0-0)	0(0-0)	16.6(0-33.3)	16.7(0-33.3)	<0.001
Pain	0(0-16.7.7)	0(0-16.7)	0(0-16.7)	33.3(0-66.7)	<0.001
Dyspnoea	33.3(0-33.3)	66.7(33.3-66.7)	66.7(33.3-100)	50(33.3-100)	0.34
Insomnia	16.65(0-33.3)	33.3(0-50)	33.3(0-66.7)	33.3(0-100)	0.001
Constipation	0(0-0)	0(0-33.3)	0(0-16.7)	33.3(0-16.65)	<0.001
Diarrhoea	0(0-0)	0(0-0)	0(0-33.3)	0(0-33.3)	<0.03
Financial impact	0(0-0)	0(0-33.3)	0(0-0)	0(0-33.3)	0.01

score 0-1 (p=0.02)] (Table 5.5). Figure 5.2 shows patient survival stratified by PG-SGA score at baseline.

Discussion

Nutritional issues are prevalent and often not appreciated among patients with AL amyloidosis.[163] BMI is relatively easy to calculate but its use in isolation has become relatively obsolete in the face of increasing global obesity.[178] In this study <4% patients had a BMI <20 and there was no correlation between BMI and PG-SGA score. Weight loss is another marker of malnutrition but it is subjective, often approximate and does not take into account the kinetics of that loss or presence of oedema or fluid retention.[179] Use of other objective parameters of nutritional status (anthropometric, biochemical and immunological) to assess nutritional state is also questionable since non-nutritional factors may affect these parameters.[180]

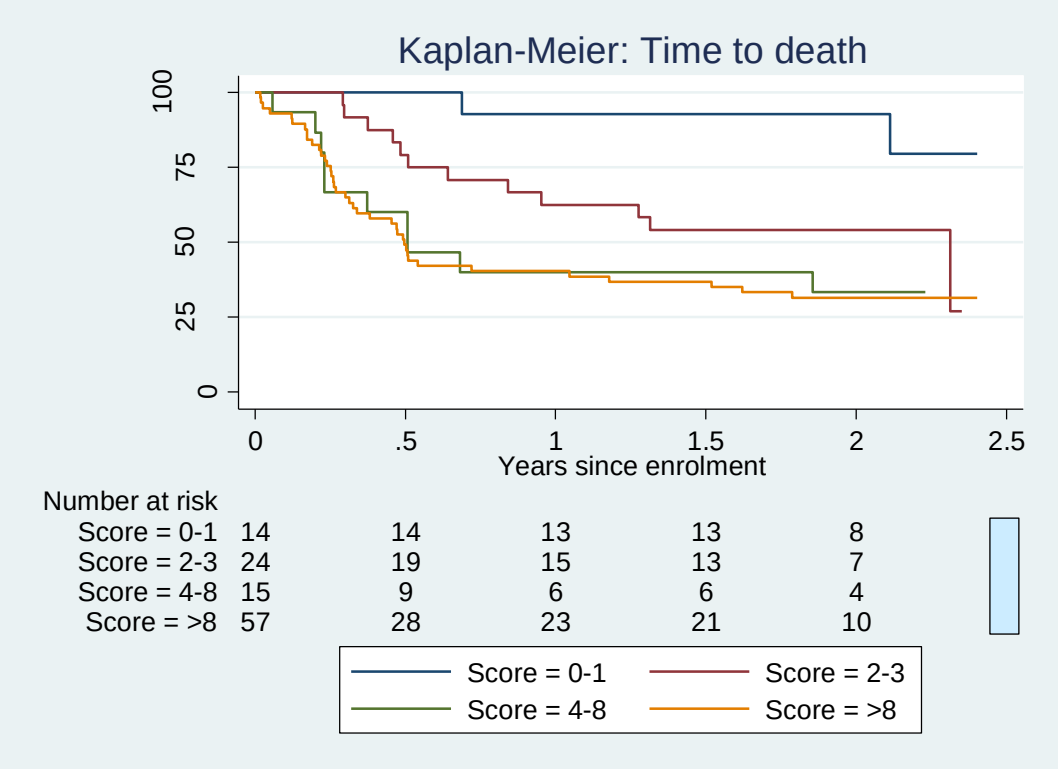
The PG-SGA includes both subjective and objective assessments of symptoms as well as physical features suggestive of malnutrition, and is quick and easy to implement, making it attractive as a nutrition assessment tool.[181] Symptoms impeding food intake such as appetite loss, nausea and early satiety, all of which affected patients in this study, are included in the assessment. Since improvement in nutritional status is unlikely unless such symptoms can be reversed, PG-SGA data may well aid appropriate nutritional intervention in such patients.

Several amyloid-related factors were associated with malnutrition among patients in this study including multisystem organ involvement. Both abnormal alkaline phosphatase and Mayo stage were also independently associated with malnutrition suggesting a possible contribution from hepatic and cardiac amyloid (causing hepatic congestion from fluid overload) in the case of the former and cardiac and renal infiltration by amyloid in the case of the latter. Hepatic AL amyloid infiltration,

Table 5.5 Factors at baseline associated with risk of death in AL amyloidosis patients.

Characteristic	Univariable		Multivariable	
	Hazard ratio (95% CI)	**P-value**	**Hazard ratio (95% CI)**	**P-value**
PG-SGA				
0-1	Ref		Ref	
2-3	4.47 (1.00, 20.03)	0.05	3.34 (0.67, 16.72)	0.14
4-8	8.13 (1.77, 37.32)	0.007	6.17 (1.18, 32.42)	0.03
≥9	8.77 (2.11, 36.49)	0.003	6.37 (1.35, 30.15)	0.02
CKD stage				
4-5 versus 1-3	1.33 (0.74, 2.37)	0.35		
Mayo				
Stage 1	Ref		Ref	
Stage 2	1.71 (0.63, 4.68)	0.30	0.94 (0.32, 2.75)	0.91
Stage 3	3.60 (1.42, 9.13)	0.007	1.66 (0.59, 4.68)	0.34
ALP (IU/l)				
>129 versus ≤129	1.04 (0.62, 1.73)	0.89		
PNS	1.13 (0.49, 2.62)	0.78		
ANS	1.44 (0.75, 2.76)	0.28		
Number organs				
≥3 versus 1-2	1.81 (1.06, 3.07)	0.03	0.99 (0.54, 1.80)	0.97

Figure 5.2 Kaplan-Meier estimates of survival from presentation according to PG-SGA score at presentation (p=0.001).



typically associated with a large amyloid burden, has previously been reported to cause weight loss.[35] Cardiac cachexia is a well-known complication of heart failure from a variety of causes,[182] and might be expected in association with an amyloid cardiomyopathy.[163] It should be noted however that the specific causes of a raised alkaline phosphatase were not ascertained in this study. These include potential contributions from a low vitamin D level and bone-derived alkaline phosphatase, as well as a raised alkaline phosphatase from a hepatic source. Reduced dietary intake and renal disease may contribute to deficiencies of vitamin D and may be assessed via food diaries and assay of serum 25-hydroxyvitamin D, respectively. Alkaline phosphatase isoenzyme analysis may help differentiate hepatic from bone-derived alkaline phosphatase.

This study demonstrates a strong association between malnutrition and poor QOL among patients with AL amyloidosis. This association was consistent across almost all individual parameters of the EORTC QLQ-C30, as well as the global QOL score. An association between malnutrition and poor quality of life has previously been demonstrated for a wide range of malignant conditions[175,183] as well as certain non-malignant diseases.[184]

The relationship between malnutrition and survival has been studied in malignant[185] and non-malignant disease.[186] Among patients with AL amyloidosis enrolled into this study, PG-SGA score at baseline was independently associated with survival. Interestingly it was the only significant parameter in a multivariable model incorporating, amongst other parameters, the Mayo staging system, which has been well documented as a strong prognostic indicator in systemic AL amyloidosis.[112] The dominance of cardiac amyloid, which the Mayo staging system is a measure of, and its impact on prognosis in AL amyloidosis is evident from data on patient deaths secondary to AL amyloidosis from the Italian amyloidosis centre (Pavia), of which 75% were

attributable to cardiac amyloid.[187] One possible explanation for the potency of the PG-SGA as a prognostic tool is that it might also take into account other factors not accounted for by the Mayo staging system. Such factors might include autonomic neuropathy and hepatic amyloid, both of which are recognised as firstly causing weight loss[188] and furthermore negatively impacting upon survival.[32,155] They were each independently and significantly associated with higher PG-SGA scores in this study.

The mechanisms of malnutrition in systemic diseases are poorly understood, but are likely to be multifactorial. Abnormalities of carbohydrate, protein and lipid metabolism have been described in malnutrition and result in loss of lean body mass and fat.[189,190] Increased energy expenditure secondary to hypermetabolism culminates in progressive wasting.[191] In cancer patients, tumour-associated metabolic abnormalities frequently prevent restoration of muscle mass by nutritional provision. Malnutrition then evolves into a cancer-driven cachexia due to complex interactions between pro-inflammatory cytokines (e.g. interleukin-1, interleukin-6) and host metabolism.[192] Several of the factors impeding food intake in the present study, such as nausea and early satiety could be the result of such metabolic changes. Cancer therapies themselves can have a significant effect on nutritional indices via a number of mechanisms such as nausea, vomiting, anorexia and a hypercatabolic state.[193] This depends upon the specific cytotoxic drug, dose, duration, and general health of the patient. Furthermore, a poor nutritional state can affect pharmaco-kinetics of cytotoxic drugs, which exacerbates toxicity and reduces tumour response to drugs.[194] Although these aspects were not formally evaluated in the present study, it is quite feasible that similar mechanisms contribute to progressive malnutrition in patients with AL amyloidosis. Early diagnosis and intervention against malnutrition are therefore imperative.

The current study provides a platform upon which to base future studies of nutritional intervention in AL amyloidosis. It would be useful to determine whether nutritional status affects tolerability and toxicity of treatment and hence treatment response. Furthermore, whether malnutrition continues to impact on QOL after patients have completed chemotherapy for AL amyloidosis and whether nutritional intervention can result in improvement in QOL and survival needs to be studied. The PG-SGA is potentially a good tool for examining this question since it provides a score which is a continuous variable unlike other nutritional assessment tools, which are often categorical. On serial analysis therefore a change in PG-SGA score may demonstrate a subtle change in nutritional status, which may not be apparent on assessment with categorical tools.[177]

Conclusion

In summary, prospective use of the PG-SGA, in 110 newly-diagnosed unselected consecutive patients with AL amyloidosis showed a strong association between malnutrition and poor QOL and reduced overall survival. The American Society of Oncology recommends that cancer treatments should be evaluated according to patient-derived outcomes such as QOL rather than clinician-derived outcomes such as tumour and biomarker response rates.[195] This study validates an assessment tool, with which to examine whether nutritional intervention in patients with AL amyloidosis influences the course of the disease or the toxicity and efficacy of therapy.

Chapter 6

Renal AA amyloidosis associated with inflammatory bowel disease

Introduction

Systemic AA amyloidosis is the second commonest form of systemic amyloidosis in the developed world. It may occur in association with any kind of chronic inflammatory condition of which inflammatory bowel disease (IBD) is one. The amyloid precursor protein in AA amyloidosis is the N-terminal fragment of the acute phase reactant serum amyloid A protein (SAA), which is synthesised in the liver.

The clinical phenotype and natural history of AA amyloidosis in IBD has largely been reported in case reports and small series. The current study focuses on amyloid burden, organ dysfunction, disease progression and the relationship of these features to biochemical markers of inflammation. There is a further detailed analysis of these features in a sub-group of patients who had renal transplants after reaching end-stage renal disease (ESRD).

Methods

The study included 26 patients followed prospectively at the NAC between 1989 and 2010. Diagnosis of AA amyloidosis was confirmed in 25 patients by histology and in 1 patient by non-invasive means. Inclusion criteria were amyloid deposition on SAP scintigraphy; evidence of chronic inflammation on the background of histologically confirmed inflammatory bowel disease; no mutations in the genes encoding transthyretin, fibrinogen A-α chain, apolipoprotein AI, apolipoprotein AII, and

lysozyme (to rule out hereditary forms of amyloidosis);[67] a negative serum free light chain assay and negative serum and urine immunofixation to rule out AL amyloidosis.[27]. A biopsy was performed if any of these criteria were not met.

Patients attended the NAC for their initial diagnostic evaluation and were followed at regular (6 monthly to annual) intervals for evaluation of organ function and monitoring of whole body amyloid load by serial ^{123}I-SAP scintigraphy. Serial electrocardiography and echocardiography to evaluate for cardiac amyloid was also performed. Blood samples for SAA and C-reactive protein (CRP) analyses were obtained at a frequency of 1-3 monthly between visits to assess levels of inflammation. Listing for renal transplantation (RTx) and transplant protocols in those patients who had reached ESRD was dependant on local transplant centre practice.

Results

Baseline characteristics of inflammatory bowel disease

Baseline characteristics of IBD in the 26 patients are listed in Table 6.1. Of these patients, 22 (14 male) had Crohn's disease (CD) and 4 (2 male) had ulcerative colitis (UC). The median (range) age of onset of CD was 23 (9 - 62). Fourteen of the patients with CD had a Crohn's colitis, 10 patients had ileal CD and 6 patients more extensive small intestinal disease. Fifteen patients with CD had intestinal resections; nine had large intestinal resections, 4 had small intestinal resections and 2 had both. Suppurative features occurred in 10 patients with CD – a fistula in 1 case, abscesses in 3 cases and a combination of the two in 5 cases. Six of 8 patients with abscesses had perianal abscesses with one patient requiring a defunctioning colostomy for such. The 4 patients with UC were diagnosed at the ages of 23, 33, 57 and 61. They had no suppurative

Table 6.1(a) Baseline characteristics of patients – IBD and systemic amyloidosis

Patient	Age at diagnosis of IBD/ CD or UC	Site of IBD[a]	Suppurative complications[b]	Extra-intestinal complications[c]	Surgical Treatment of IBD	Medical Treatment of IBD[d]	Age of diagnosis of amyloidosis	SAP scintigraphy - Extra-renal amyloid[e]	Dead (D) or Alive (A)/ Age at census or of death
1	62/CD	I	IA	O		U	67	S	A/78
2	33/CD	I,C		AS		S, A, ASA	53	S	D/55
3	11/CD	S,C	F - Ileorectal	G	Colectomy and ileostomy, small intestinal resections	S, A, T	31	S/A	A/36
4	33/CD	I		EP, G,A	Right hemicolectomy	S, A, T	61	S/A	A/66
5	53/CD	I			Ileal resection	S, A, ASA	70	S	D/71
6	47/CD	C				S, ASA	63	S	A/69
7	16/CD	C		EN	Panproctocolectomy	A	36	S/A	A/47
8	30/CD	S,C			Panproctocolectomy	U	44	S/A	D/44
9	19/CD	S,I	IA F – enteroenteric		Ileal resection	U	37	S/A	D/37
10	21/CD	S	PA, F - perianal		Small intestinal resections	S, A, T	54	S/A/L	D/58
11	12/CD	C			Colectomy and ileorectal anastomosis	A, ASA, T	29	S/A	A/33
12	54/CD	S,C				S, A	54	S/A	A/56
13	24/CD	C	PA, F - perianal		Panproctocolectomy	S, A	34	S	A/38

[a] - I=ileum S=small intestine C-colonic; [b] – IA=intra-abdominal abscess PA=perianal abscess F=Fistulae; [c] – O=oxalate stones AS=ankylosing spondylitis G=gallstones A=IBD-related arthropathy EP=episcleritis EN=erythema nodosum I=iritis M=oral ulcers; [d] – A=azathioprine ASA=5-aminosalicylates 6-MP – 6-Mercaptopurine S=steroids M=methotrexate T=anti-TNF α agents C=cyclosporine E=elemental diet U=unknown; [e] –S=spleen A=adrenal L=liver

Table 6.1(b) Baseline characteristics of patients – IBD and systemic amyloidosis.

Patient	Age at diagnosis of IBD/CD or UC	Site of IBD[a]	Suppurative complications[b]	Extra-intestinal complications[c]	Surgical Treatment of IBD	Medical Treatment of IBD[d]	Age of diagnosis of amyloidosis	SAP Scintigraphy – extra-renal amyloid[e]	Dead (D) or Alive (A)/ Age at census or of death
14	22/CD	C		I,AS		S, ASA	29	S/A	D/30
15	12/CD	I	PA			S, T	25	S/A	A/28
16	30/CD	C	PA	M	Defunctioning colostomy	A, 6-MP, T	50	S	A/50
17	24/CD	I		AS	Small intestinal resections	S, A, ASA, M	36	S	A/48
18	18/CD	S,C	PA, F - perianal	G	Panproctocolectomy and ileostomy, Small intestinal resections	U	38	S	A/49
19	18/CD	I,C	PA, F - perianal	EN	Right hemicolectomy , sigmoid colostomy	S, A, C	34	S/A	A/47
20	9/CD	I,C		EN	Right hemicolectomy	S, A, E	32	S/A	A/57
21	24/CD	C			Panproctocolectomy and ileostomy	ASA	25	S	A/60
22	15/CD	I		EN,M		S, ASA	22	S/L	D/39
23	61/UC	C		AS		S	77	S	D/78
24	57/UC	C			Panproctocolectomy	-	64	S	D/67
25	23/UC	C				S,ASA	26	S	A/33
26	33/UC	C		AS		S, ASA	63	S/L	A/70

[a] - I=ileum S=small intestine C-colonic; [b] – IA=intra-abdominal abscess PA=perianal abscess F=Fistulae; [c] – O=oxalate stones AS=ankylosing spondylitis G=gallstones A=IBD-related arthropathy EP=episcleritis EN=erythema nodosum I=iritis M=mouth ulcers; [d] – A=azathioprine ASA=5-aminosalicylates 6-MP – 6-Mercaptopurine S=steroids M=methotrexate T=anti-TNF α agents C=cyclosporine E=elemental diet U=unknown; [e] –S=spleen A=adrenal L=liver

features and only one of them (Patient 24; Table 6.1) needed surgical intervention. She had a panproctocolectomy.

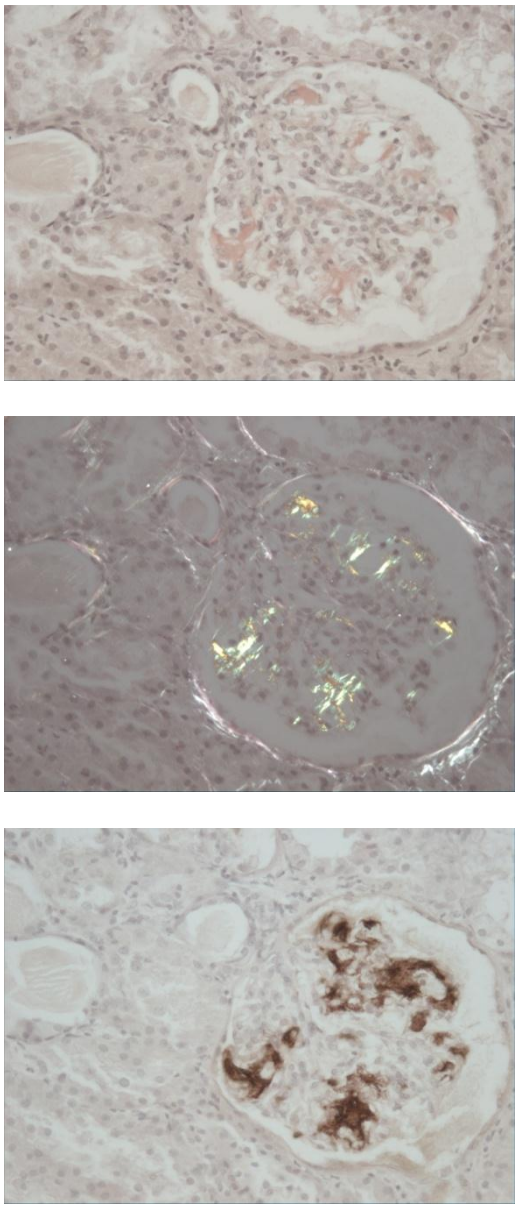
Extra-intestinal manifestations of IBD occurred in 11 of the 22 patients with CD. Ocular complications (iritis, uveitis and episcleritis) occurred in 3 cases, erythema nodosum in 4 cases and oral apthous ulceration in 2 cases. In 4 patients with small intestinal disease there was evidence of gallstone disease (3 cases) and renal oxalate stones (1 case). Ankylosing spondylitis occurred in 3 patients and another 2 had a peripheral arthropathy attributed to CD. The only extra-intestinal manifestations in the 4 patients with UC was ankylosing spondylitis in 2 cases.

Baseline characteristics of systemic amyloidosis

In all cases the diagnosis of IBD pre-dated the diagnosis of systemic amyloidosis. The histological diagnosis of amyloid was made from renal biopsies in 23 cases, an appendicectomy specimen in 1 case and an intestinal resection specimen in 1 case. Immunohistochemistry with anti-SAA antibodies was positive in all cases (Figure 6.1). Only 1 patient (Patient 2; Table 6.1) did not have a histological diagnosis of amyloid but she had unequivocal renal and splenic amyloid on her SAP scan typical of AA amyloidosis, proteinuric renal dysfunction, a background of chronic inflammation associated with CD, no evidence of a plasma cell dyscrasia and no genetic mutations recognised in causing any of the known forms of hereditary systemic amyloidosis.

The median (range) time from diagnosis of CD to diagnosis of systemic amyloidosis was 16.3 years (0.3-33.2). In the 4 patients with UC the time from diagnosis of UC to diagnosis of systemic amyloidosis was 3.1, 7.0, 16.0 and 29.9 years. All of the patients in the current series had impaired renal function and/or evidence of proteinuria at the time of diagnosis. This was the presenting clinical feature in all except Patient 16, who was referred by a cardiologist because he had echocardiographic

Figure 6.1 Renal biopsy (glomerulus) with AA amyloid deposition - with positive Congo-red uptake (top), red-green birefringence when the same section is viewed under cross-polarised light (middle) and immunohistochemical staining with a monoclonal antibody confirming the presence of serum amyloid A protein within the deposits (bottom).



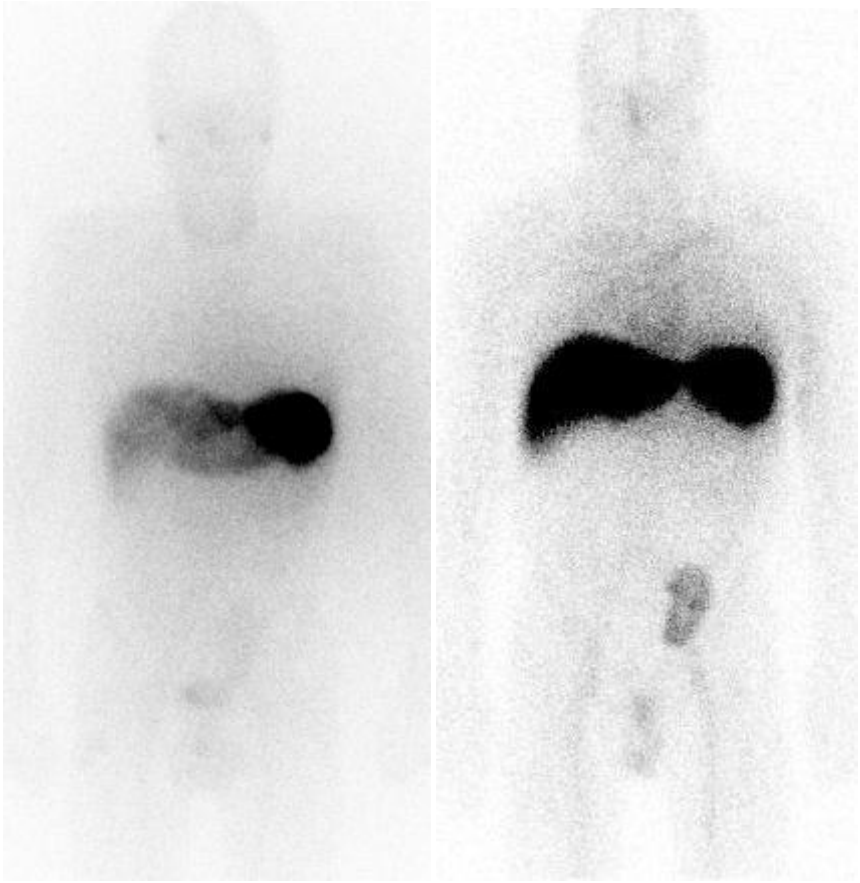
features of cardiac amyloid when he was investigated for non-specific chest pain. At the time of his diagnosis he had SAP scintigraphic evidence of renal and splenic amyloid, proteinuric renal dysfunction, AA-type amyloid on a renal biopsy and neither evidence of a clonal disorder nor possession of a genetic mutation that might encode for a hereditary form of amyloidosis. Ten patients had already reached ESRD requiring renal replacement therapy prior to their first review at the NAC, 3 of whom had already undergone RTx.

In addition to renal amyloid there was evidence of splenic amyloid in all patients on SAP scintigraphy at their baseline visits (Figure 6.2). In 3 patients there was also evidence of hepatic amyloid on SAP scintigraphy. These patients did not have hepatomegaly and had normal liver synthetic function. Thirteen patients had evidence of adrenal amyloid on SAP scintigraphy. Two of these patients had Addison's disease but of note both had had long-term prior steroid use. One further patient, who had taken prolonged steroid courses, also had Addison's disease but he did not have adrenal amyloid on SAP scintigraphy.

Clinical course of systemic amyloidosis

The cohort of 26 patients, which included 6 patients with CD who had RTx, were followed for a median (range) time of 4.1 (0-20.0) years. In 5 patients, who did not require RTx (Patients 4, 7, 11, 13 and 26; Table 6.1) there was improvement in proteinuric renal dysfunction with complete resolution of nephrotic-range proteinuria over 5.6, 9.0, 3.5, 4.3 and 6.6 years respectively. They had median serial SAA values of 12, 3, 5, 10 and 7 mg/l and median CRP values of 2, 2, 2, 5 and 5 mg/l respectively during these periods. In keeping with their normal or very mildly elevated inflammatory markers they had well-controlled symptoms of IBD.

Figure 6.2 Serial anterior whole body SAP scintigraphy of Patient 22 after renal transplantation - hepatic and splenic amyloid with no evidence of renal graft amyloid in the left iliac fossa 6.9 years after transplantation (left) and progression of hepatic amyloid and development of graft amyloid 8.1 years after transplantation (right).



All of the other 21 patients had evidence of progressive renal dysfunction and/or progressive proteinuria. During follow-up in addition to the 10 patients, who presented to the NAC after having reached ESRD there were 5 further patients who progressed to ESRD during follow-up. The median time from proteinuric renal dysfunction to ESRD by Kaplan – Meier estimate in the entire cohort was 6.3 years. The median (range) rate of renal decline in the entire cohort was eGFR loss -9 (-294 to +7) ml/min/year. Five patients (Patients 3, 12, 15, 17 and 19; Table 6.1), who had progressive renal dysfunction had serial blood tests over >6 months (median (range) period of 62.4 (8.2-155.3) months) to assess inflammation prior to ESRD or RTx. Their median SAA values were 46, 16, 25, 18 and 26 mg/l respectively and their median CRP values were 25, 8, 6, 1 and 13 mg/l respectively during the respective periods. The potential lack of correlation between SAA and CRP is particularly apparent in the case of Patient 17 in whom serial CRP values were consistently 1 mg/l despite consistently elevated SAA levels.

Five patients (Patients 11, 12, 13, 18 and 24; Table 6.1) had hypotensive/hypovolaemic episodes which precipitated rapidly progressive renal dysfunction. Patients 12, 18 and 24 rapidly progressed to ESRD after developing sepsis requiring hospitalisation. Patient 12 had normal renal function despite proteinuria prior to hospital admission. Patients 11 and 13 were diagnosed with renal amyloid after a significant and acute deterioration in renal function from a baseline of mild renal dysfunction, after surgery for their IBD. Subsequent tight inflammatory control in both cases resulted in resolution of proteinuria.

There was no evidence of a peripheral or autonomic neuropathy in any patient during follow-up. Patient 16 was the only patient who had echocardiographic evidence of cardiac amyloid during follow-up.

Outcomes of renal transplantation

Six patients had RTx after reaching ESRD (Table 6.2). Four patients had deceased-donor transplants and 2 patients had live-related transplants. The median (range) time from ESRD to RTx in this group of patients was 1.7 (0.2 – 7.8) years. Four grafts (Patients 17, 18, 19 and 20; Table 6.2) were functioning at census 0.8, 3.2, 4.2 and 20.1 years after RTx. There was no evidence of amyloid recurrence in these grafts on serial SAP scans over the course of 0.8, 2.5, 3.2 and 19.5 years after RTx, respectively. Furthermore there was no evidence of proteinuric renal dysfunction in any of these patients during follow-up. One graft (Patient 22; Table 6.2) failed 14.5 years after RTx with evidence of amyloid recurrence in the graft on SAP scintigraphy more than 6 years before graft failure. Another patient (Patient 21; Table 6.2), whose graft had survived 24.6 years, was being considered for a pre-emptive second live-related renal transplant, as he was approaching ESRD at census. There had been neither evidence of recurrent graft amyloid on his serial SAP scans nor evidence of significant proteinuria after RTx to suggest ongoing amyloid deposition in the graft. His progressive renal dysfunction was attributed to recurrent urolithiasis.

The median serial SAA and CRP values after RTx in the 6 transplant recipients are outlined in Table 6.2. In 4 patients (Patients 18, 19, 20 and 21; Table 6.2) the median SAA and CRP values during follow-up, which of note was more than 20 years in Patients 20 and 21, were in single figures and there was no evidence of graft amyloid recurrence in any of their grafts. In the 2 other patients (Patients 17 and 22; Table 6.2) the median serial SAA values after RTx were 19 mg/l and 40 mg/l respectively and the median serial CRP values were 8.5 mg/l and 5 mg/l respectively. The potential discrepancy in the levels of these 2 acute phase reactants is again highlighted here. The former patient's graft had no evidence of amyloid recurrence at census but he had only

Table 6.2 Characteristics of patients with AA amyloidosis secondary to CD who had RTx.

Patient	Time from ESRD to RTx (yrs)	Type of graft[a]	Median SAA/CRP (mg/l) after RTx	Recurrence of graft amyloid on SAP scan[b]/ Time of recurrence after RTx (yrs) or time of last SAP scan with no evidence of recurrence after RTx (yrs)	Graft failure[c]/ Time from RTx to graft failure or census (yrs)	Dead (D) or Alive (A)/ Time from RTx to census or death (yrs)
17	2.5	L	40/5	N/0.8	N/0.8	A/0.8
18	7.3	D	4.5/1.5	N/2.5	N/3.2	A/3.2
19	7.8	D	3/3	N/3.2	N/4.2	A/4.2
20	0.2	L	7/2	N/19.5	N/20.1	A/20.1
21	1	D	5/3	N/22.2	N/24.6	A/24.6
22	0.3	D	19/8.5	Y/8.1	Y/14.5	D/16.1

a – L=live-related graft D=deceased donor graft; b and c – Y=yes N=no

been followed for 0.8 years after RTx and the latter's graft failed because of recurrent amyloid 14.5 years after RTx.

Survival

During follow-up, which was over a median (range) period of 4.1 (0-20) years, 9 patients in the cohort died at a median (range) age of 55 (30-75). The median survival by Kaplan-Meier estimate from diagnosis of amyloid was 16.9 years. Two patients, both of whom required renal replacement therapy, died from myocardial infarction, 2 died from pneumonia, 1 from intestinal perforation and peritonitis and 1 post-operatively after cardiac valve replacement. In the 3 remaining cases the causes of death were not known.

Discussion

The association between IBD and amyloidosis was first described in 1936.[196] It has evolved from a post-mortem diagnosis[197] into a pre-mortem diagnosis.[198] This is probably due to an increased appreciation of this association together with improvements in diagnostic techniques for amyloid including better histological diagnosis with immunohistochemistry and SAP scintigraphy.[199]

There have been several studies estimating the incidence of systemic amyloidosis associated with IBD. In the USA this incidence has been estimated as 0.9% in CD and 0.07% in UC.[200] In Northern Europe the incidence of systemic amyloidosis secondary to CD has been estimated as 0.8% with a ten-fold increase in risk compared to UC.[201] These incidence studies, which are more than a decade old, may not be relevant to the present day. Results from one epidemiological study of CD from Leiden University Hospital suggested that the incidence of amyloidosis secondary

to CD is falling.[202] This could in part be because of improved treatments, especially with the addition of biologic therapies to the treatment armamentarium.

The current study is not a study of incidence but there is a very obvious discrepancy in the number of referrals of patients with CD compared to UC to the only amyloidosis centre in the UK, which corroborates the results from other studies.[200,201] One possible explanation for this is the larger acute phase response seen in CD compared to UC,[203] which may be explained by the higher prevalence of suppurative features in the former. In the current study 10 of 23 patients had fistulae and/or abscesses. Fifteen of 22 cases of CD in the study of Greenstein et al.[200] and 13 of 18 cases in the study of Wester et al.[201] also had similar features. Another possible explanation for the apparent discrepancy in risk of development of amyloidosis between CD and UC is that whereas surgery can be a definitive treatment in UC by completely removing the inflammatory source, as in Patient 24 who had complete resolution of nephrotic-range proteinuria after panproctocolectomy, in CD as the entirety of the gastrointestinal tract can be affected surgery may not be as efficacious in reducing inflammation. It is also worth highlighting that the extra-intestinal manifestations of IBD in isolation can provide a significant inflammatory stimulus. Ankylosing spondylitis, for example, which occurred in two of the patients with UC, is recognised in its own right as causing AA amyloidosis.[204]

The fact that all patients in the current cohort had proteinuric renal dysfunction highlights the fact that AA amyloidosis is a disease of the kidneys. It should be noted however that there are a number of other potential causes of renal dysfunction in patients with IBD. These include interstitial nephritis secondary to 5-aminosalicylates, glomerulonephritis, renal oxalate stones and proximal tubular defects.[205] Furthermore those patients with severe IBD are at risk of sepsis and surgery, both of which may

result in hypotensive episodes and hence precipitate acute tubular necrosis, which can contribute to chronic renal impairment.

Hepatic amyloid was evident in 3 patients on SAP scintigraphy in the current series but was of no clinical significance with respect to liver synthetic function. Hepatic amyloid in the context of AA amyloidosis in a SAP scintigraphic study at the NAC was found to confer a reduced survival risk.[42] Two of the 3 cases with hepatic amyloid in the current series died, 4 and 17 years after diagnosis of amyloid. It is probably indicative of a large amyloid burden in the setting of AA amyloidosis. Only one patient in the current study had echocardiographic features of cardiac amyloid. He was awaiting an endomyocardial biopsy at census. This feature is unusual in the context of AA amyloidosis. At the NAC only 2 of 374 patients with AA amyloidosis followed over 15 years till 2005 had echocardiographic evidence of cardiac amyloid.[39]

The current series highlights the benefits of good inflammatory control in systemic AA amyloidosis associated with IBD. There is evidence that if serial SAA values are maintained below 10 mg/l in inflammatory conditions causing AA amyloidosis then one can achieve amyloid regression and improvement of amyloidotic organ function and improved survival.[41] This is highlighted in those patients in the current series in whom complete resolution of proteinuria was achieved when median SAA values were mainatined $\leq$12 mg/l during follow-up. The case of Patient 17 highlights the importance of monitoring serial SAA levels rather than CRP levels in patients with IBD and systemic AA amyloidosis. His CRP levels were consistently 1mg/l during follow-up, which in isolation could give the sense of good inflammatory control. His serial SAA levels (median value 18mg/l) were however disproportionately high, indicative of ongoing inflammation, and as a result he had progressive renal dysfunction secondary to ongoing amyloid deposition, which culminated in ESRD.

The benefits of inflammatory control in terms of improvement of amyloidotic organ dysfunction are not dependant on type of medical treatment administered or whether surgical resection of the inflammatory source is pursued but on how efficacious a given intervention is in reducing SAA levels. In this series however those patients in whom complete resolution of proteinuria was achieved had significant lengths of diseased intestine resected, resulting in reduction of inflammation. Fifteen of 22 patients with CD (68%) had some form of intestinal surgery in this series. This percentage, although of a small number of patients, is more than the 43% from one epidemiological study of CD from Wales over a similar time period.[206] Intestinal surgery in CD is warranted with severe disease and hence is probably a surrogate marker of inflammatory risk and the risk of developing amyloidosis. It is worth highlighting that this series spans more than 20 years. Treatments have changed over this time period including availability and use of biologic therapy, which has revolutionised treatment of IBD. Furthermore, some drugs such as 5-aminosalicylates, which were used as the mainstay of treatment in some of the patients with CD in this series are now considered to be less effective as anti-inflammatory drugs in CD compared to UC.[207]

A feature of amyloidotic kidneys, which was apparent in this cohort is the risk of decompensation in the face of sepsis, hypotension or other physiological stresses. This phenomenon occurred in 5 cases, three of whom had surgery prior to acute deterioration of renal function and three of whom progressed rapidly to ESRD. This risk would give further weight to careful evaluation for proteinuric renal dysfunction with regular urine dipstick analysis in patients with IBD and ongoing inflammation. A positive dipstick should prompt investigation for systemic AA amyloidosis. If systemic amyloidosis is confirmed there should be tight control of inflammation guided by serial SAA values, aggressive treatment of sepsis and careful hydration during and after surgery.

The graft and patient survival in all patients who had RTx after reaching ESRD in the current series was excellent and appears quite comparable to RTx in other renal diseases (http://www.uktransplant.org.uk). There was only 1 graft failure, 14.5 years after RTx in Patient 22, who was also the only patient in the transplanted group to die during follow-up, 16.1 years after RTx. His graft failed because of ongoing inflammation and recurrent graft amyloid, interestingly with a time lag of more than 6 years between SAP scintigraphic evidence of recurrent graft amyloid and subsequent graft failure. During follow-up, like Patient 17, he had disproportionately high serial SAA levels when compared to serial CRP levels. It would appear feasible to recommend RTx in selected patients with IBD who have reached ESRD secondary to AA amyloidosis. There should be tight control of inflammation, as assessed by serial SAA levels, in tandem with planning for RTx and this should be maintained after RTx.

Conclusion

Systemic AA amyloidosis occurs more commonly in CD than UC. This may be explained by the more pronounced acute phase response seen in CD, which may in turn be related to a higher prevalence of suppurative features in CD. AA amyloidosis in the context of IBD is a disease of the kidneys characterised by progressive proteinuric renal dysfunction. In those with well-controlled inflammation, guided by serial SAA levels rather than CRP levels, one may see amyloid regression and improvement of organ function. Amyloidotic kidneys are sensitive to physiological stresses. Therefore aggressive treatment of sepsis or hypotension in those with confirmed amyloid is imperative. In those who progress to ESRD and have RTx both graft and patient survival are good. To prevent graft amyloid recurrence it is important that there is tight inflammatory control, again guided by serial SAA values.

Chapter 7

Solid organ transplantation in AL amyloidosis

Introduction

Systemic AL amyloidosis may present with dysfunction of a single organ or alternatively there may be amyloid deposition and dysfunction of multiple organ systems concomitantly. Deposition of amyloid in the kidneys presents with varying degrees of proteinuric chronic kidney disease (CKD) and may lead to end-stage renal disease (ESRD). AL amyloid deposits in the heart typically cause a restrictive cardiomyopathy and diastolic dysfunction. Once CHF has supervened in AL amyloidosis, prognosis is poor with a median survival of 4-6 months.[28] Hepatic AL amyloid can present indolently with hepatomegaly or deranged liver function tests or occasionally with liver failure or hepatic rupture,[208] and is usually associated with presence of extensive extra-hepatic deposits.[42] Hyperbilirubinaemia in the context of hepatic AL amyloid confers a particularly poor prognosis of <4 months.[35]

Despite considerable advances in chemotherapy in recent years leading to improved median survival times,[187] a significant proportion of patients have advanced, unsalvageable organ dysfunction by the time they are diagnosed with systemic AL amyloidosis, and the majority of patients continue to die from their disease. Furthermore, patients with advanced cardiac and hepatic involvement are usually too unwell at presentation to tolerate the doses of chemotherapy that are required to successfully suppress their underlying clonal disease.

The role of solid organ transplantation in AL amyloidosis is contentious due to shortage of donor organs. Previous small series have shown inferior outcomes with heart[209] and kidney transplantation among patients with AL amyloidosis compared to

those with other causes of cardiac and renal failure, respectively. Deaths have been associated with both recurrence of amyloid in the graft or progressive deposition of amyloid in non-transplanted organs. This study presents the clinical management and outcome in 45 patients with AL amyloidosis over a 25 year period, who were selected to receive solid organ transplants, and highlights in particular, the disease-related and treatment-related factors that are likely to influence outcome with solid organ transplantation during the modern era of effective chemotherapy.

Methods

All patients who underwent renal, cardiac or liver transplantation for organ failure secondary to AL amyloidosis between 1984 and 2009 were identified from the UK National Amyloidosis Centre (NAC) database. Only patients without multiple myeloma underlying their AL amyloidosis received solid organ transplants. Additionally, selection criteria for renal and cardiac transplantation included absence of extensive disease outside the failing organ. A good ECOG performance status was required for renal transplantation in contrast to cardiac transplantation, where performance status prior to the procedure was poor. Patients who underwent orthotopic liver transplantation were those with a poor prognosis due to extensive liver amyloid associated with clinical decompensation. Among patients with systemic AL amyloidosis followed at the NAC during the 25 year period, the 45 patients presented here were not the only cases who fulfilled the above criteria; listing for solid organ transplantation was also dependent upon attitudes to transplantation in AL amyloidosis within each transplant centre, which varied substantially.

The diagnosis of amyloidosis was confirmed histologically in all cases. Among cases where amyloid was confirmed histologically but immunohistochemical staining with antibodies against all known amyloid fibril proteins excluded AA amyloidosis but

was non-diagnostic of AL type, the following criteria were required for inclusion; evidence of a clonal B cell dyscrasia; absence of amyloidogenic mutations in the genes encoding transthyretin, fibrinogen A α-chain, apolipoprotein AI, apolipoprotein AII and lysozyme (to rule out hereditary forms of amyloidosis);[67] absence of a chronic inflammatory disorder.

Assessment at the baseline NAC visit, repeated 6 to 12 monthly, included clinical evaluation, detailed biochemical tests of renal and hepatic function, serum and urine immunoelectrophoresis, electrocardiographic and echocardiographic studies. Serial whole body anterior and posterior scintigraphic imaging was undertaken to establish baseline and change in whole body amyloid load, as previously described. Organ involvement by amyloid was determined according to international consensus criteria.[114] Baseline haematological investigations included bone marrow aspirate, trephine and skeletal survey. Serum free light chains (FLCs) were prospectively followed at 1-3 monthly intervals in all patients after January 2002 and, prior to this date, were measured retrospectively from stored sera obtained during all visits to the NAC. A complete clonal response (CR) following chemotherapy was defined as no evidence of a monoclonal protein on serum and urine immunoelectrophoresis and normalisation of the FLCs in the context of normal renal function or normalisation of the FLC ratio in the context of renal impairment. No response (NR) was defined as a reduction of less than 50% of the serum paraprotein or the amyloidogenic FLC. A partial hematologic response (PR) following chemotherapy was defined as any response not fulfilling the criteria for CR or NR.

Pre-operative transplant assessments and post-operative immunosuppression regimens were according to local protocols in each case. Different regimens of chemotherapy and/or stem cell transplantation were administered at different times in relation to solid organ transplantation in each of the patient groups.

Results

Liver transplantation

Nine patients, all of whom had a dominant hepatic presentation of amyloidosis, received orthotopic liver transplantation (OLT) for systemic AL amyloidosis (Table 7.1). Three cases had underlying lambda light chain secreting plasma cell dyscrasias and 6 had an amyloidogenic kappa secreting clone. Median (range) age at diagnosis of amyloidosis was 56 (range 29-66) years and median (range) time from symptom onset to diagnosis of amyloidosis was 2.3 (0-10.6) months. Median (range) time from diagnosis to OLT was 5.5 (1.5-10.5) months. Pre-OLT SAP scintigraphy revealed a large total body amyloid load in all cases. Patients were followed for a median (range) of 0.9 (0-12.5) years from OLT.

One and 5-year patient survival from transplantation among those receiving OLT was 33% and 22% respectively. Causes of death among the six patients who died within the first year were as follows: intraoperative death due to cardiac decompensation (1 case), sepsis (3 cases), sudden unexplained death (1 case) and declining renal function (1 case).

Three patients (5, 7 and 9; Table 7.1) were too unwell to receive chemotherapy at any point during the course of their illness and died within 5 months of OLT. The remaining 6 patients received chemotherapy, including stem cell transplantation after OLT in 3 cases (Patients 1, 2 and 8; Table 7.1). Two patients without extra-hepatic organ dysfunction at the time of OLT, both of whom subsequently underwent and responded to stem cell transplantation (Patients 1 and 2), were alive at census 12.5 and 5.7 years after OLT without evidence of graft failure despite asymptomatic recurrence of liver amyloid. Among the 4 remaining cases to receive chemotherapy, 3 died within 2 years of OLT despite achieving a clonal

Table 7.1 Characteristics and outcome among 9 patients with systemic AL amyloidosis who underwent liver transplantation

Patient No/ [a]Sex	Age at symptom onset	[b]Extra-hepatic organ involvement at presentation by consensus criteria	Time from diagnosis of amyloid to OLT (months)	[c]Treatment pre-OLT/ treatment post-OLT	[d]Clonal response to treatment	Timing of clonal response relative to OLT (months)	Clonal relapse after treatment (Yes/No)	Recurrence of graft amyloid by SAP scintigraphy	Timing of amyloid recurrence or last SAP scan after OLT with no evidence of recurrence (months)	[e]Dead / Alive	Time from OLT to census or death (years)
1 (M)	54	N	6	N/S	CR	+60	N	Y	31	A	12.5
2 (M)	44	N	9	N/C+S	PR	+17	N	Y	7	A	5.7
3 (M)	61	N	5	C/C	PR	+10	N	Y	10	D	0.9
4 (M)	31	N	6	C/N	NR	-	-	N	11	D	0.9
5 (M)	66	K,H,A	2	N/N	N/A	-	-	N	3	D	0.4
6 (F)	63	K,H	8	N/C	CR	+24	N	N	24	D	2
7 (F)	56	H	1	N/N	N/A	-	-	-	-	D	0
8 (F)	29	N	10	C/S	PR	-4	N	-	-	D	0.4
9 (F)	60	G	2	N/N	N/A	-	-	-	-	D	0.3

[a] - M=Male F=Female
[b] - N=None, K=Kidneys, H=Heart, A=Autonomic nervous system, G=Gastrointestinal tract
[c] - N=None, C=Chemotherapy, S=Stem cell transplantation
[d] - NR=No response, PR=Partial response, CR = Complete Response, N/A = Not applicable
[e] - A=alive, D=dead

response with chemotherapy (Patients 3, 6 and 8). The remaining case who did not achieve a clonal response to chemotherapy (Patient 4) died 0.9 years after OLT.

Four patients (Patients 1 to 4) developed rapidly progressive proteinuria following OLT associated with pre-existing renal amyloid deposits. Interestingly, this occurred even in patients 1 and 2, despite good clonal responses to chemotherapy. Patient 1, who had a clonal CR with stem cell transplantation, underwent cadaveric renal transplantation 9.4 years after OLT. His CKD had been thought to be multifactorial from amyloid, hypertension and immunosuppression. There was no evidence of recurrent renal amyloid in the graft by SAP scintigraphy 2.5 years after renal transplantation, despite deterioration of renal allograft function to an eGFR of 29 ml/min at the time of census.

Renal transplantation

Twenty-two patients, all of whom presented with renal dysfunction, received renal transplants (19 deceased donor and 3 live donor) after reaching end-stage renal failure secondary to AL amyloidosis (Table 7.2). Twelve patients had an underlying lambda light chain secreting plasma cell dyscrasia and 10 had an amyloidogenic kappa clone. Median (range) age at diagnosis of amyloid was 54 (41-68) years and median (range) time from symptom onset to diagnosis was 5.8 (0-58.2) months. Median (range) time from diagnosis to renal transplantation was 55.0 (12.3-204.6) months and from commencement of dialysis to renal transplantation was 26.7 (0.8-98.3) months. Median (range) follow-up from renal transplantation was 4.8 (0.2-13.3) years. Only 3/22 patients had amyloidotic extra-renal organ dysfunction at the time of renal transplantation; of the liver (Patient 4; Table 7.2), heart (Patient 17) and nerves (Patient 21) in one case each. Seventeen of 22 patients had SAP scintigraphy before renal

Table 7.2(a) Characteristics and outcome among 22 patients with systemic AL amyloidosis who underwent renal transplantation.

Patient No / [a]Sex	Age at symptom onset	[b]Extra-renal organ involvement at RTx	Time from diagnosis of amyloid to RTx (months)	[c]Chemotherapy treatment pre-RTx / post-RTx	[d]Clonal response to treatment	Timing of clonal response relative to RTx (months)	[e]Clonal relapse after initial (1st line) chemotherapy	Timing of clonal relapse relative to RTx (months)	[f]Graft failure / time from RTx to failure (years)	Dead (D) / Alive (A)	Time from RTx to census or death (years)
1 (F)	40	N	84	S/N	CR	-75	N		N	A	4.7
2 (M)	56	N	52	C+S/N	PR	-42	N		N	A	2
3 (F)	41	L	68	C/C	PR	-63	Y	-9	N	A	7.6
4 (F)	62	N	12	N/C	NA		NA		N	D	5.8
5 (F)	50	N	39	C/C	NA		NA		Y/13.1	D	13.3
6 (F)	46	N	55	C/N	PR	-42	N		N	A	6.3
7 (F)	61	N	66	N/C	PR	79	N		N	D	7.5
8 (M)	57	N	32	C+S/N	PR	-24	Y	50	N	A	5.3
9 (F)	51	N	57	C+S/N	CR	-50	N		N	A	7.5
10 (M)	57	N	55	C/N	PR	-44	N		N	D	0.2
11 (F)	60	N	115	C/N	CR	-85	Y	28	N	A	2.7

[a] - M= male F= female; [b] - N=none L=liver H=heart P=peripheral neuropathy A=autonomic neuropathy; [c]- N=none S=stem cell transplantation C=chemotherapy; [d] - NA=not applicable NR=no response PR=partial response CR=complete response; [e]- Y=yes N=no NA=not applicable; [f] - Y=yes N=no

Table 7.2(b) Characteristics and outcome among 22 patients with systemic AL amyloidosis who underwent renal transplantation.

Patient No / [a]Sex	Age at symptom onset	[b]Extra-renal organ involvement at RTx	Time from diagnosis of amyloid to RTx (months)	[c]Chemotherapy treatment pre-RTx / post-RTx	[d]Clonal response to treatment	Timing of clonal response relative to RTx (months)	[e]Clonal relapse after initial (1st line) chemotherapy	Timing of clonal relapse relative to RTx (months)	[f]Graft failure / time from RTx to failure (years)	Dead (D) / Alive (A)	Time from RTx to census or death (years)
12 (M)	49	N	30	C/C	NA		NA		N	D	6.5
13 (M)	45	N	160	C/N	PR	-138	N		N	A	3.9
14 (M)	54	N	66	C/N	PR	-48	N		N	A	4.7
15 (F)	43	N	204	C/C	NA		NA		N	D	5.3
16 (F)	66	N	28	C/N	NR		NR		N	D	4.8
17 (F)	65	H	61	N/N	NA		NA		N	D	1.9
18 (F)	53	N	62	C/C	NA		NA		N	A	9.8
19 (F)	58	N	48	C+S/N	PR	-30	N		N	D	1.3
20 (F)	41	N	123	C/N	NA		NA		Y / 0.9	D	2.8
21 (M)	45	P, A	32	C/C	PR	-25	Y	3	N	A	1.3
22 (M)	63	N	51	C/C	PR	-45	N		N	A	0.5

[a] - M= male F= female; [b] - N=none L=liver H=heart P=peripheral neuropathy A=autonomic neuropathy; [c]- N=none S=stem cell transplantation C=chemotherapy;

[d] - NA=not applicable NR=no response PR=partial response CR=complete response; [e]- Y=yes N=no NA=not applicable; [f] - Y=yes N=no

transplantation with extra-renal amyloid deposits evident by this technique in all but one such case.

There were no perioperative deaths. By Kaplan-Meier analysis, median estimated patient survival from diagnosis was 13.0 years, from dialysis was 9.1 years and from renal transplantation was 6.5 years. Among 10 patients who died, cause of death was sepsis (6 cases), gastrointestinal haemorrhage (1 case; Patient 20), cardiac decompensation (1 case; Patient 10) and was unknown in 2 cases. The patient who died of cardiac failure did not have echocardiographic features of cardiac amyloidosis pre-operatively but was discovered at autopsy to have coronary vessel amyloid in the absence of myocardial infiltration.

Nineteen patients received chemotherapy or SCT before renal transplantation to try and halt amyloid deposition and thereby prevent progressive impairment of their native amyloidotic kidneys. Acute irreversible kidney injury and dialysis dependence associated with hypotension was precipitated in 4 of 5 patients who underwent SCT (Patients 1, 2, 8 and 19), the remaining case (Patient 9) being dialysis-dependent prior to SCT. Due to the lack of a pre-chemotherapy FLC sample, 4/19 patients were not evaluable for a clonal response. Among 15 evaluable patients, 14 had a clonal response to treatment (11 PR, 3 CR) and 1 (Patient 16) had NR. All of the complete responders (Patients 1, 9 and 11) were alive at census, with 2 cases (Patients 1 and 9) maintaining a clonal CR for 10.9 and 11.7 years respectively from 1st line treatment. Three patients (Patients 4, 7, 17) in whom the diagnosis of AL amyloidosis was not confirmed prior to renal transplantation, did not receive chemotherapy before renal transplantation. Patient 17 did not receive chemotherapy at any point. Ten patients received chemotherapy after renal transplantation, 8 of whom had received chemotherapy earlier in the course of their disease. No transplant failed due to recurrent amyloid despite evidence of amyloid within the renal allografts of 5 patients (Patients 5, 7, 12, 14 and 18) detected by SAP

scintigraphy a median (range) of 5.6 (4.4-7.8) years from renal transplantation. Two grafts failed, one from chronic allograft nephropathy (Patient 5) and one from scarring related to recurrent transplant pyelonephritis (Patient 20). One case (Patient 18) developed proteinuria from graft amyloid 6.2 years after renal transplantation but this resolved after successful chemotherapy with preservation of renal allograft function at census 3.6 years later.

Cardiac transplantation

Fourteen patients, 13 of whom presented with advanced cardiac failure and 1 of whom developed cardiac amyloidosis during follow-up for gastrointestinal amyloid, received cardiac transplants (Table 7.3). Eleven cases had an underlying lambda light chain secreting plasma cell dyscrasias and 3 cases had an amyloidogenic kappa clone. Median (range) age at diagnosis of amyloidosis was 52 (38 to 58) years and median (range) time from symptom onset to diagnosis was 10.0 (1.0-25.1) months. Median (range) time from diagnosis to cardiac transplantation was 6.3 (0-73.6) months. Median follow-up from cardiac transplantation was 4.4 (0-10.1) years. At the time of cardiac transplantation, 8 patients had no extra-cardiac amyloid according to international amyloid consensus criteria; 4 had amyloidotic dysfunction of 1 other organ and 2 patients had dysfunction of 2 other organs.

Median survival from cardiac transplantation by Kaplan-Meier analysis was 7.5 years for the entire cohort. There were 2 peri-operative deaths (Patients 12 and 13; Table 7.3) one from left ventricular failure and another from high right-sided cardiac pressures, shown at autopsy to be due to pulmonary amyloid. Among 8 patients who underwent SCT after cardiac transplantation, median survival from cardiac transplantation was 9.7 years compared to 3.4 years among cases that did not undergo

Table 7.3 Characteristics and outcome among 14 patients with systemic AL amyloidosis who underwent heart transplantation.

[a]Patient No / Sex	[b]Age at symptom onset	[c]Extra-cardiac amyloid Pre-HTx (Consensus criteria)	Diagnosis to HTx (months)	[d]Chemotherapy treatment pre-HTx / post-HTx	[e]Clonal response to treatment	Time to clonal response relative to HTx (months)	[f]Clonal relapse after initial treatment	Timing of clonal relapse relative to HTx (months)	[g]Recurrence of amyloid by echo / Time to recurrence (months)	Dead (D) / Alive (A)	Time from HTx to census or death (years)	[h]Cause of death
1 (M)	58	N	9	C/C+S	PR	-7	Y	18	Y / 97	A	9	N/A
2 (F)	59	N	10	C/C+S	PR	-1	Y	15	N	A	4.7	N/A
3 (M)	52	P	32	C/S	PR	-13	N		N	A	2.2	N/A
4 (F)	57	N	1	N/S	CR	19	N		N	A	1.8	N/A
5 (M)	43	K	2	N/S	CR	5	N		N	D	10.1	SCD
6 (M)	51	K	5	N/C	CR	43	N		Y / 28	D	9.7	IC
7 (M)	51	N	8	N/S	PR	23	Y	82	Y / 82	D	9.7	PCA
8 (M)	55	N	5	N/C+S	CR	30	Y	83	Y / 83	D	7.5	PCA
9 (M)	U	N	0	N/C	NR				U	D	4.6	PEA
10 (F)	51	K, P	26	C/N	PR	-16	Y	17	N	D	4.1	PEA
11 (F)	50	K, L	9	N/C	NR				N	D	2.7	PEA
12 (M)	51	G	74	C/N	PR	-65	Y	-1	N	D	0.0	PF
13 (M)	46	N	2	N/N	N/A				N/A	D	0.0	LA
14 (M)	38	N	3	N/C+S	PR	4	N		Y / 23	D	2.8	PCA

a - M=Male F=Female; b and g – U=unknown; c – N=none P=peripheral neuropathy K=kidneys G=gastrointestinal tract L=liver; d – N=none C=chemotherapy S=stem cell transplantation; e –N/A=not applicable NR=no response PR=partial response CR=complete response; f and g – Y=yes N=No; h – N/A=not applicable SCD=sudden cardiac death IC=ischaemic colitis PCA=progressive cardiac amyloidosis PEA=progressive extra-cardiac amyloidosis PF=pump failure LA=lung amyloid

SCT (P=0.01). No patient who underwent SCT after cardiac transplantation had major extra-cardiac organ dysfunction at the time of SCT.

All patients received chemotherapy during the course of their disease apart from the single case (Patient 13; Table 7.3), who died peri-operatively. Five patients were fit enough to receive chemotherapy before cardiac transplantation (Patients 1, 2, 3, 10 and 12) and they all achieved a partial haematological response. All but 3 cases (Patients 10, 12 and 13) received chemotherapy after cardiac transplantation, including SCT in 8 patients.

Amyloid recurred in the cardiac allografts of 5 patients (Patients 1, 6, 7, 8, 14), all of whom had persistence or relapse of their haematological disease, and was first detected a median of 82.6 (22.6-96.8) months from cardiac transplantation. Four of these 5 cases died and a further 3 cases died from progressive extra-cardiac amyloid (Patients 9, 10, 11). There was a single unexplained sudden cardiac death in a patient who remained in haematological CR (Patient 5) which was not felt to be associated with progressive amyloidosis.

Discussion

This is the largest series of both cardiac and renal transplantation for systemic AL amyloidosis and reports the first cohort of patients with decompensated hepatic AL amyloidosis to undergo OLT. Outcomes among OLT recipients were poor, in marked contrast to outcomes following heart and kidney transplantation which, in this highly selected and monitored group of patients, were relatively good.

Despite recent advances in management of AL amyloidosis,[72,187] many patients continue to present with advanced, irreversible amyloidotic end-organ damage and nearly 30% die within 1 year of diagnosis.[187] The role of solid organ transplantation in AL amyloidosis remains contentious due to concerns about recurrence within the graft

and progressive disease outside the graft. Most series of renal[210] and cardiac transplantation[209] in amyloidosis however, pre-date the discovery that systemic chemotherapy which successfully suppresses monoclonal light chain production can halt ongoing AL amyloid deposition.[27,76] Some published transplant series even include patients with unknown or multiple amyloid types.[211,212]

The patients reported here were carefully selected for their transplant procedure. Selection criteria for cardiac transplantation included advanced cardiomyopathy, age under 60 years, and absence of myeloma or extensive extra-cardiac amyloidosis. On the basis of the significantly prolonged survival among patients who received SCT after cardiac transplantation in this series, compared to those who did not, there is evidence that only patients who are predicted to be fit enough to receive SCT after the cardiac transplant procedure should be listed for cardiac transplantation. Patients with advanced amyloid cardiomyopathy, are usually too unwell to tolerate aggressive chemotherapy before cardiac transplantation, and given their predisposition to sudden cardiac death, should probably be listed for urgent cardiac transplantation with a view to subsequent SCT. Selection criteria for renal transplantation included ESRD, age under 70 years, absence of myeloma or extensive extra-renal amyloidosis, ECOG performance status of 1 or 2, and, wherever possible, sufficient suppression of the underlying plasma cell dyscrasia by chemotherapy to prevent ongoing amyloid accumulation according to serial SAP scans. Importantly, only 3/22 patients in this series were in clonal CR prior to renal transplantation although they had nearly all received chemotherapy and achieved at least a clonal PR. The evidence from this series, like other series,[213] is that chemotherapy should usually be administered prior to consideration of renal transplantation in systemic AL amyloidosis. Although reasonable outcomes have previously been reported with renal transplantation followed by autologous stem cell transplantation in a small number of patients with AL amyloidosis,[214] it is apparent from

experiences at the NAC that stem cell transplantation and/or chemotherapy is associated with a significant risk of irreversible renal allograft dysfunction. Selection criteria for OLT included decompensated hepatic AL amyloidosis, age under 70 years and absence of clinically significant cardiac involvement by amyloid. Analogous to recipients of cardiac allografts, patients with decompensated liver disease are usually too unwell to receive chemotherapy prior to OLT, and the patients reported in the current series were scheduled to receive chemotherapy or SCT after OLT.

OLT was associated with poor outcomes, mainly due to presence of extensive extra-hepatic amyloid,[215] which, even if clinically silent at the time of OLT, usually caused sufficient organ dysfunction after the transplant procedure to compromise administration of chemotherapy aimed at halting ongoing amyloid deposition. In this series 4/9 (44%) OLT recipients were too unwell to receive chemotherapy at any stage after the transplant, all of whom died within 1 year of the procedure. Among 5/9 patients who did receive chemotherapy after OLT, only two were alive at census including one who developed subsequent ESRD, the remaining 3 deaths occurring 0.4, 0.9 and 2 years after OLT. The 1- and 5-year patient survival following OLT of 33% and 22% respectively in this series compares very poorly to all-cause OLT survival estimates which exceeds 87% and approaches 75% respectively in the USA[216] and 82% and 71% respectively in Europe (http://www.eltr.org), and, in the context of donor organ shortages, does not encourage consideration of OLT in systemic AL amyloidosis generally.

Outcomes with renal transplantation in this cohort were good. Interestingly, no renal allografts failed from recurrent amyloid despite the fact that only 3 patients achieved a clonal CR with chemotherapy. Two of ten deaths in renal transplant recipients were related to extra-renal amyloid deposits. There is evidence in this series that renal transplantation should generally be offered only to AL patients with a

preserved ECOG performance status and little or no clinically significant extra-renal amyloidosis, who have achieved at least a clonal PR with prior chemotherapy. One area of contention is whether dialysis-dependent patients without extra-renal amyloidosis should receive chemotherapy whilst on dialysis, the sole purpose of which is to achieve a clonal response in order to permit listing for renal transplantation, or whether such patients should undergo renal transplantation followed by chemotherapy/SCT to prevent ongoing amyloid deposition.[214] This series, which reports the best outcomes of any to date, supports attempting to achieve a clonal response prior to renal transplantation. The median patient survival of 6.5 years from renal transplantation is impressive considering the patients were transplanted over the course of 15 years and included 3 cases in whom the diagnosis of AL amyloidosis was unknown at the time of renal transplantation. In addition, the median patient survival from dialysis of 9.1 years in these 22 patients is distinctly better than the 3.6 years from dialysis in non-transplanted AL amyloidosis ESRD patients at the NAC,[70] although much of this difference is likely to reflect selection bias.

Survival among patients presenting with advanced cardiac AL amyloidosis in the absence of cardiac transplantation remains dismal.[112] Up to one third of patients with AL amyloidosis die within 12 months of diagnosis, frequently from cardiac involvement.[187] Furthermore, even among patients who survive for longer than 12 months, quality of life is generally poor with persistent and severe limitation of physical activity. The median patient survival from cardiac transplantation of 9.7 years among those who received subsequent SCT in this series is comparable to US 'all-cause' cardiac transplant survival.[217] Successful outcomes among patients with dominant and isolated cardiac AL amyloidosis who receive sequential cardiac and stem cell transplantation have been widely reported in recent years.[218,219] There is evidence that this approach should be adopted by cardiac transplant units worldwide. Although

successful outcomes have been reported in small numbers of patients with chemotherapy followed by cardiac transplantation,[220] cumulative evidence would favour urgent cardiac transplantation followed by SCT in such cases due to the risks of chemotherapy in patients with advanced cardiac amyloidosis. Whenever there is likely to be a substantial delay before the cardiac transplant however, careful administration of high dose dexamethasone or bortezomib, both of which have the potential to induce a rapid clonal response whilst preserving the option of harvesting stem cells in the future, should be considered.

Conclusion

In summary, this series provides support for continued cardiac and renal transplantation for carefully selected patients with AL amyloidosis, but shows that OLT for decompensated hepatic AL amyloidosis, is probably not an appropriate use of donor organs which continue to be in short supply. Solid organ transplantation in AL amyloidosis should always be accompanied by chemotherapeutic strategies to halt ongoing amyloid production.

Chapter 8

Hepatic amyloidosis – A ten-year biopsy study (2000-2009)

Introduction

Hepatic amyloid is frequently present in patients who develop systemic amyloidosis and may occur without derangement of liver function tests and without symptoms.[42] It is most commonly seen in AL amyloidosis[42] where it might present indolently with hepatomegaly or deranged liver function tests or occasionally as liver failure[221] or hepatic rupture.[208] Those patients with a dominant hepatic presentation of AL amyloidosis have historically been deemed to have a poor prognosis, particularly in the context of hyperbilirubinaemia. In one study of patients followed prior to the year 2000 a bilirubin >34 μmol/l was associated with survival of only 1 month.[35] Patients with systemic amyloidosis may present with dominant hepatic features where a diagnosis of amyloid is made unexpectedly by liver biopsy. This study outlines the clinical features, treatment strategies and outcomes in such patients presenting over a ten year period from 2000 to 2009 at the NAC.

Aims

The first aim of this study was to try and provide a diagnostic algorithm for gastroenterologists should a diagnosis of amyloid be made unexpectedly by liver biopsy in patients presenting with deranged liver functions tests and/or hepatomegaly. The second aim was to evaluate the role of chemotherapeutic treatments in the modern era of treatment in those patients with dominant hepatic AL amyloidosis to see if a survival

benefit could be conferred in historically what has been a group that has been perceived to have a poor prognosis.[35]

Methods

Patients

Between January 2000 and November 2009 all patients in whom amyloid was diagnosed unexpectedly by liver biopsy, were identified from the database at the NAC. Typing of amyloid was supported by immunohistochemical analysis of biopsies, evaluation for a plasma cell dyscrasia with serum and urine electrophoresis and the serum free light chain assay and assessment of inflammation with CRP and SAA analyses. Exclusion or confirmation of hereditary systemic amyloidosis was achieved by genetic sequencing of genes known to encode for variant proteins with amyloidogenic potential.[67] Specific sampling by laser microdissection (LMD) and tandem mass spectrometry (MS)-based proteomic analysis was used to characterise amyloidosis secondary to Leucocyte cell-derived chemotaxin 2 (LECT-2).[109]

Assessment at the baseline NAC visit, repeated 6 to 12 monthly, included clinical evaluation, detailed biochemical tests of renal and hepatic function, assessment of serum and urine immunoelectrophoresis, serum free light chain analysis, electrocardiographic and echocardiographic studies. Whole body anterior and posterior scintigraphic imaging was undertaken to establish whole body amyloid load. Organ involvement was classified according to the international amyloid consensus criteria in those patients with AL amyloidosis.[114]

Different regimes of chemotherapy and/or stem cell transplantation were administered to those patients with AL amyloidosis according to protocols at local treatment centres, if they were deemed fit enough to tolerate treatment. Serial

serum-free light chains (FLC) were determined in all patients after 2003 and retrospectively sought by analysis of stored sera, if available, to elucidate treatment responses to chemotherapeutic agents. Healthy, polyclonal FLC concentrations rise progressively through advancing stages of chronic kidney disease (CKD),[222] 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 lately been validated in myeloma.[223] The FLC response to chemotherapy was defined as the percentage of the dFLC at baseline that remained following treatment. Serial SAA levels, to assess adequacy of anti-inflammatory treatment in those patients with AA amyloidosis, were monitored at a frequency of 1-3 monthly.

Statistical analysis

Patient follow-up was censored in November 2009, and in the single patient who was lost to follow-up, at their last clinic visit prior to this date. Kaplan-Meier analyses and Cox proportional hazards regression were used to investigate factors associated with overall survival of patients with AL amyloidosis. To validate use of dFLC for calculation of response to chemotherapy in AL amyloidosis, time-updated dFLC response was included as a co-variate in the survival analysis. All factors which were of statistical significance ($p<0.05$) in univariate analyses and/or clinical significance were included in multivariate analysis. Cut points were chosen by their clinical relevance.

Results

Patient characteristics

Ninety-three patients had a dominant hepatic presentation of systemic amyloidosis with amyloid identified unexpectedly by liver biopsy. Eighty-two biopsies were via the percutaneous route, 7 were transjugular biopsies and 4 were obtained at laparoscopy or laparotomy. Eighty-four patients (55 male) had systemic AL amyloidosis (Table 8.1). Nine patients had other forms of systemic amyloidosis – systemic AA amyloidosis (2 patients), TTR-type (ATTR) amyloidosis (3 patients), hereditary apolipoprotein AI (AApoAI) amyloidosis (3 patients), and LECT-2 amyloidosis (1 patient) (Table 8.2).

Systemic AL amyloidosis - baseline characteristics

Of the 84 patients with systemic AL amyloidosis, 2 patients (2%) had no evidence of a clonal disorder on serum or urine analysis. The diagnosis of AL amyloidosis in these 2 patients was based upon immunohistochemical analysis, clinical features and exclusion of hereditary forms of amyloidosis. Sixty-one cases (73%) had evidence of an abnormal monoclonal protein on serum or urine electrophoresis and 73/80 cases (91%) had evidence of a light chain excess at presentation. The median (range) age of diagnosis was 61 years (30-84). Involuntary weight loss had occurred in 65 patients (77%) by the time of diagnosis. The presenting symptomatology was typically non-specific and included lethargy in 40 patients (48%), abdominal discomfort in 20 (24%), appetite loss in 26 (33%), nausea in 21 (25%) and early satiety in 24 (29%) patients. The median (range) time from symptom onset to diagnosis was 0.7 years (0-2.9). At the time of diagnosis 83 patients (99%) had hepatomegaly, 21 (25%) were jaundiced and 10 (12%) had ascites. No patient was encephalopathic at diagnosis.

Table 8.1 Baseline laboratory markers in AL amyloidosis patients.

Test	Patient number	Patient frequency	Median (Range)	Normal values
Bilirubin (µmol/l) <19 ≥19	84	 44 (52.4%) 40 (47.6%)	16.5 (5, 401)	<19
Alkaline phosphatase (U/l) <516 (4 times upper limit of normal) ≥516	84	 36 (42.9%) 48 (57.1%)	560 (90, 3891)	35-129
Gamma-glutamyl transferase (U/l) <62 ≥62	78	 1 (1.3%) 77 (98.7%)	604.5 (47, 5837)	8-61
Aspartate aminotransferase (U/l) <55.5 (1.5 times upper limit of normal) ≥55.5	84	 44 (52.4%) 40 (47.6%)	49 (17, 223)	<37
Alanine aminotransferase (U/l) <41 ≥41	78	 35 (44.9%) 43 (55.1%)	43 (12, 231)	<41
Platelets ($x10^9$/l) <401 ≥401	84	 58 (69.1%) 26 (31.0%)	344.5 (132, 820)	140-400
CKD Stage 0-2 3-5	84	 44 (52.4%) 40 (47.6%)	2 (0, 5)	
Albumin (g/l) <35 ≥35	84	 43 (51.2%) 41 (48.8%)	34 (15, 56)	35-50
Prothrombin time (seconds) ≤16 >16	79	 53 (67.1%) 26 (32.9%)	15.2 (11.6, 28.3)	12-16
Proteinuria (g/24 hours) <0.5 0.5-3 >3	81	 23 (28.4%) 36 (44.4%) 22 (27.2%)	1.3 (0, 11.4)	<0.15

Table 8.2 Characteristics of non-AL amyloidosis patients.

Patient number	Sex[1]	Amyloid Type[2] (mutation)	Histological localisation of amyloid	Age of diagnosis	Organ involvement[3]	SAP scan – total body amyloid load (organs involved)[4]	Bilirubin at diagnosis (µmol/l)	ALP at diagnosis (U/l)	AST at diagnosis(U/l)	Alive (A) or Dead (D) /Age at census or death
1	M	AA	Parenchyma	74	K/L	L (L,S,K)	13	748	29	A/82
2	F	AA	Parenchyma	42	K/L	L (L,S,K)	6	105	48	A/43
3	F	ATTR (V122I)	Vascular	82	H	None	33	154	63	A/85
4	M	ATTR (V122I)	Vascular	79	H	None	15	290	29	D/80
5	M	ATTR (T60A)	Vascular	69	H	None	12	203	26	A/75
6	M	AApoAI (G26R)	Parenchyma	55	K/L	M (L/S)	15	290	54	A/60
7	F	AApoAI (G26R)	Parenchyma	50	K/L	S (L/S)	12	552	104	A/56
8	M	AApoAI (G26R)	Vascular	46	K/L	S (S)	6	463	49	D/51
9	M	LECT-2	Vascular	52	K	S (S)	12	68	25	A/55

[1]Sex : M=male, F=female

[2]Amyloid type : AA = Systemic AA amyloidosis, ATTR = Hereditary transthyretin-related amyloidosis, LECT-2 = Leucocyte cell-derived chemotaxin-2 amyloidosis

[3]Organ involvement : K=kidney, H=heart, L=liver

[4]SAP scan – total body amyloid load : L=large, M=moderate, S=small (Organs involved on SAP scan : L=liver, S=spleen, K=kidney)

The baseline haematological and biochemical parameters of these patients are outlined in Table 8.1. All patients had abnormal liver function tests except one who was investigated with a liver biopsy for hepatomegaly. The most common biochemical abnormality was a raised alkaline phosphatase which occurred in 80 cases (95%). The median alkaline phosphatase in this group was more than 4 times the upper limit of normal. Sixty cases (71%) had a raised aspartate aminotransferase with a median value that was just less than 1.5 times the upper limit of normal. Serum bilirubin was abnormal in 40 cases (48%) and prothrombin time was prolonged in 26/80 cases (33%). Nine patients (11%) had no evidence of renal dysfunction, 13 patients (16%) had CKD stage 1, 22 patients (27%) had CKD stage 2 and the remainder (46%) had markedly abnormal renal function with an eGFR ≤60 ml/min.

SAP scintigraphy was performed in all cases at baseline except one. It identified a large or moderate total body amyloid load in 10 (12%) and 73 (88%) cases respectively. Hepatic and splenic amyloid was universal on all SAP scans (Figure 8.1). Extra-hepatic amyloid, as assessed by the international amyloid consensus criteria, was common by the time of diagnosis, with 43 patients (51%) having ≥2 organ involvement (Table 8.3). Only 11 cases (13%) had no evidence of extra-hepatic amyloid. Among the 73 patients with extra-hepatic amyloid, the kidneys (62 cases - 85%) and heart (39 cases - 53%) were the commonest organs involved (Table 8.3).

Systemic AL amyloidosis – clinical course

Fifty-four (64%) patients died during follow-up. After one year, two years, three years, four years and five years 45% (95% CI: 35% to 56%), 62% (95% CI: 51% to 72%), 63% (95% CI: 52% to 74%), 67% (95% CI: 56% to 78%) and 70% (95% CI: 58% to 81%) patients had died respectively. The median survival by Kaplan-Meier estimate from diagnosis of amyloid in the entire cohort was 1.2 years (Figure 8.2).

Figure 8.1 Anterior-posterior whole body SAP scintigraphic view of a patient with AL amyloidosis treated successfully with chemotherapy – pre-treatment image (left) with massive hepatic and splenic amyloid with little blood pool and post-treatment image (right) showing marked regression of amyloid from the liver and spleen and a more visible blood pool accompanied by normalisation of liver function tests.

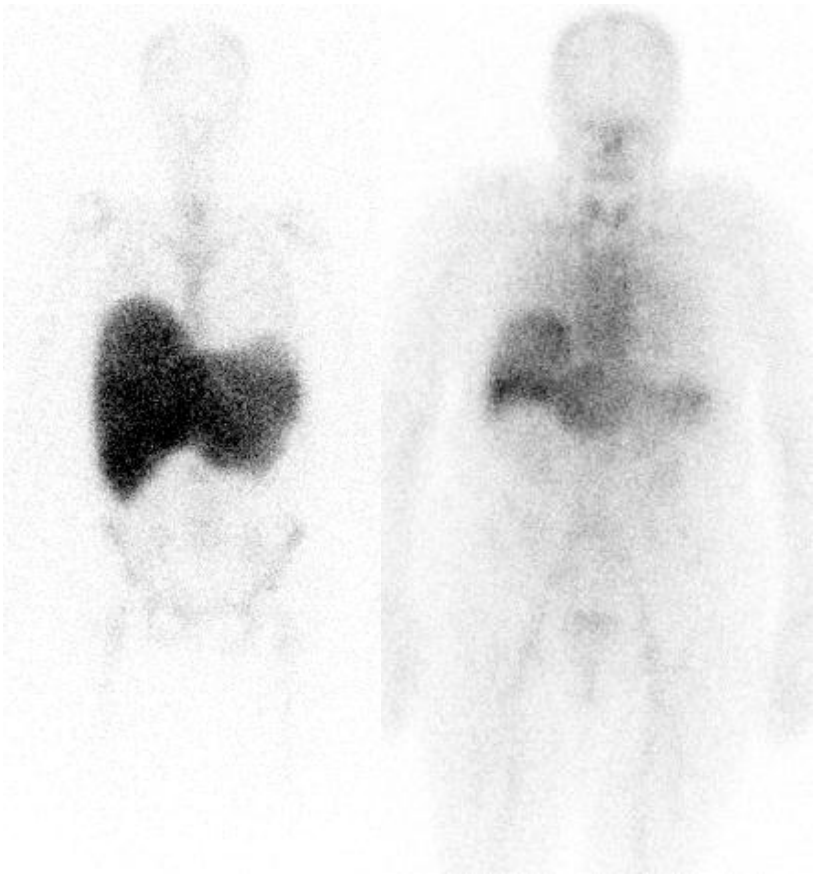
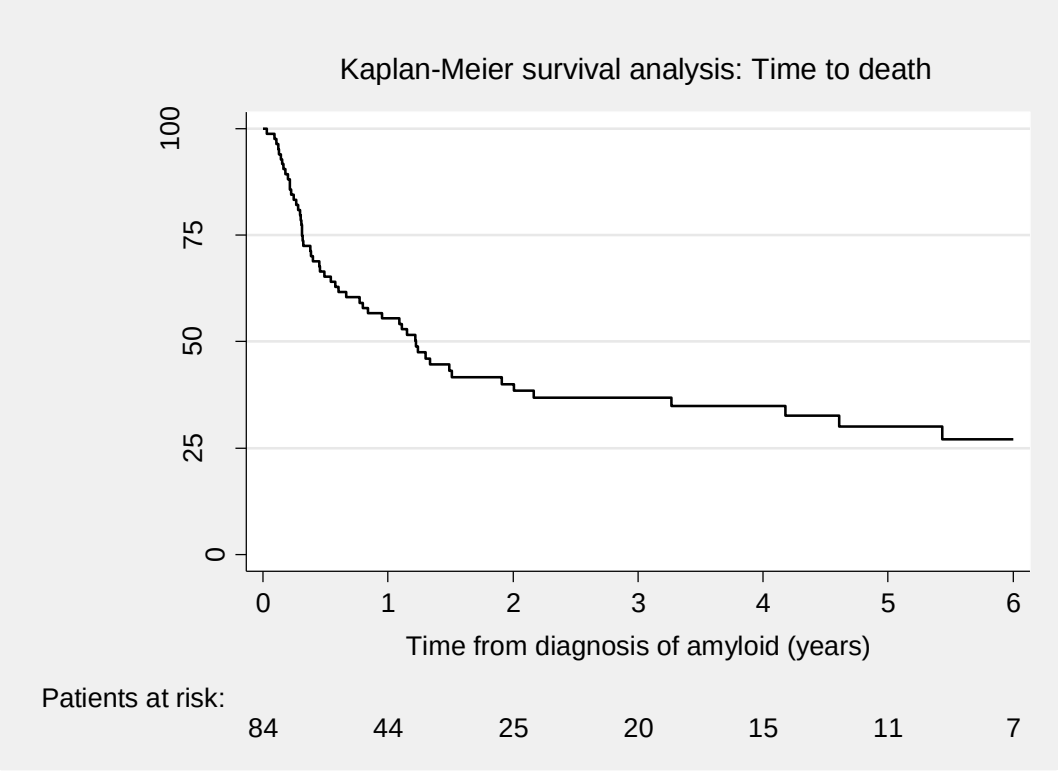


Table 8.3 Extra-hepatic organ involvement by the international amyloid consensus criteria at baseline in AL amyloidosis patients.

Organ involved	Number of patients	Percentage
Kidneys	62	84.9
Heart	39	53.4
Peripheral neuropathy	6	8.2
Autonomic neuropathy	16	21.9
Gastrointestinal tract	4	5.5
Soft tissue	6	8.2
No extra-hepatic organ	11	13.1
One extra-hepatic organ	30	35.8
Two extra-hepatic organs	29	34.5
Three or more extra-hepatic organs	14	16.7

Figure 8.2 Kaplan-Meier plot showing the time to death from biopsy diagnosis of amyloid in AL amyloidosis patients.



Chemotherapy including stem cell transplantation, was administered to 62 patients (74%), with evaluable clonal responses in 58 cases. The remainder died before treatment could be administered or were deemed too unwell to tolerate treatment.

Liver transplantation (OLT) was undertaken in 3 patients for decompensated liver disease. One patient with cardiac amyloid died intra-operatively at the age of 56 because of cardiac decompensation secondary to hypotension during surgery. Another patient was transplanted at the age of 61 and died from pseudomonal septicaemia 1.1 years after OLT. He received chemotherapy before and after OLT and had a >50% dFLC response but despite this developed progressive cardiac amyloidosis. The patient who was alive and well at census 5.5 years after OLT had a stem cell transplant after OLT and achieved a >50% dFLC response.

Factors at presentation associated with increased mortality on multivariable analysis (Table 8.4) were older age (relative risk of death, 1.69 for each additional decade [95% CI, 1.23 to 2.35];p=0.001), cardiac amyloid (relative risk of death, 2.29 if present [95% CI, 1.23 to 4.24];p=0.009), hyperbilirubinaemia (relative risk of death, 2.33 if the serum bilirubin was raised [95% CI, 1.14 to 4.77];p=0.02) and hypoalbuminaemia (relative risk of death, 3.41 if the serum albumin was low [95% CI, 1.49 to 7.83];p=0.004). There was also a clear trend between risk of death and magnitude of dFLC response to chemotherapy (relative risk of death of 0.75 [95% CI, 0.33 to 1.68;p=0.02] for patients achieving a 50-90% dFLC response and 0.15 [95% CI, 0.04 to 0.58;p=0.02] for patients achieving a >90% dFLC response, compared to patients achieving <50% dFLC response).

Systemic AA amyloidosis

Two patients, both of whom were alive at census 1.2 and 8.4 years after diagnosis, had systemic AA amyloidosis with a large total body amyloid load on SAP scintigraphy and

Table 8.4 Factors at diagnosis associated with risk of death in AL amyloidosis patients.

	Unadjusted analysis		Multivariate analysis	
Characteristic	**Hazard ratio (95% CI)**	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Age; for each 10 years older	1.39 (1.08, 1.79)	0.01	1.69 (1.23, 2.35)	0.001
Organ involvement:				
Cardiac amyloid; (Yes vs No)	2.33 (1.35, 4.01)	0.002	2.29 (1.23, 4.24)	0.009
Biochemical markers:				
Bilirubin; ≥19umol/l versus <19umol/l	2.80 (1.61, 4.87)	<0.0001	2.33 (1.14, 4.77)	0.02
Albumin; <35 g/L versus ≥35 g/L	4.25 (2.31, 7.80)	<0.0001	3.41 (1.49, 7.83)	0.004
ALP; >516 IU/L versus ≤516 IU/L	3.04 (1.68, 5.50)	<0.0001	0.91 (0.37, 2.24)	0.85
AST; ≥55.5 IU/L versus <55.5 IU/L	2.77 (1.60, 4.80)	<0.0001	1.39 (0.69, 2.83)	0.36
Other factors:				
Response to treatment*;		0.03		0.02
<50% dFLC response	Ref		Ref	
50-90% dFLC response	0.80 (0.36, 1.74)		0.75 (0.33, 1.68)	
>90% dFLC response	0.22 (0.06, 0.77)		0.15 (0.04, 0.58)	
Year of biopsy; (2005-09 versus 2000-04)	0.80 (0.46, 1.39)	0.43	0.86 (0.46, 1.59)	0.62

*Time-updated FLC response

evidence of proteinuric renal dysfunction (Table 8.2). One patient (Patient 1; Table 8.2), whose deranged liver function tests prompted a liver biopsy, developed amyloidosis on the background of chronic inflammation associated with a paraspinal abscess, which occurred after septicaemia following an intestinal perforation 30 years prior to diagnosis of amyloid. The other patient (Patient 2; Table 8.2) was co-infected with HIV and hepatitis C. She had a liver biopsy to investigate for potential hepatic fibrosis. The potential sources of inflammation in her case were recurrent skin abscesses at the sites of intravenous drug usage and bronchiectasis. With abstinence from intravenous drug usage, scrupulous treatment of infections with antibiotics and use of regular intravenous immunoglobulin therapy for bronchiectasis her serial SAA values normalised during follow-up and there was resolution of proteinuria and regression of amyloid on serial SAP scans within 1 year.

Hereditary systemic amyloidosis

Six patients, all of whom had no family history of amyloidosis, had hereditary forms of systemic amyloidosis (Table 8.2). Three of these cases had genetic mutations that encode for variant transthyretin. Two patients of Afro-Caribbean ethnicity were heterozygous for the V122I mutation (Patients 3 and 4; Table 8.2) and one patient of Irish ethnicity was heterozygous for the T60A mutation (Patient 5; Table 8.2). In all 3 cases hepatomegaly and deranged liver function tests prompted liver biopsies. Hepatic amyloid was visualised in the vasculature and not in the parenchyma in all. There was a corroborative lack of hepatic uptake on SAP scintigraphy in these patients. The deranged liver function tests and hepatomegaly in these cases, unlike in the previously described cases of the acquired forms of systemic amyloidosis, were secondary to cardiac failure and hepatic congestion on the background of an amyloid cardiomyopathy rather than hepatic parenchymal infiltration with amyloid. The diagnosis of amyloid

was quite incidental in each of these cases, as the hearts were the only organs that were functionally affected by amyloid, with no clinically significant extra-cardiac amyloid in any case. The subsequent management of these patients utilised standard heart failure treatments. One patient who was heterozygous for the V122I mutation died 1.5 years after diagnosis and the other 2 patients were alive 3 and 5.8 years after diagnosis.

Three further cases had genetic mutations that encode for variant apolipoprotein AI (Patients 6, 7 and 8; Table 8.2). In 2 of these cases, both of whom were alive at census, there was evidence of hepatic parenchymal amyloid on liver biopsy. Liver function tests were stable for 4.9 (Patient 6; Table 8.2) and 6.0 (Patient 7; Table 8.2) years after diagnosis of amyloid with no evidence of progressive amyloid deposition on serial SAP scans, suggestive of a slow turnover of amyloid. The former patient reached end-stage renal failure, which on retrospect most probably was secondary to renal amyloid and had a renal transplant before the correct diagnosis of amyloidosis was made by liver biopsy. The latter case presented with renal dysfunction before diagnosis of amyloid, which did not progress during follow-up. The third case (Patient 8; Table 8.2) had hepatic vascular amyloid and no parenchymal amyloid in his liver biopsy and like the ATTR patients in this series had no evidence of hepatic amyloid on SAP scintigraphy. His deranged liver function tests were secondary to a non-amyloid cardiomyopathy and associated hepatic congestion. He, like Patient 6, developed renal dysfunction which progressed to end-stage renal failure 4 years prior to a liver biopsy diagnosis of amyloid and died 5.1 years after this diagnosis.

LECT-2 amyloidosis

Patient 9 (Table 8.2), who was alive at census 3.5 years after diagnosis of amyloid, had LECT-2 amyloidosis. He had hepatitis C and had a liver biopsy to investigate for potential fibrotic liver disease. Amyloid was seen only in his hepatic vasculature on

histological analysis. On baseline SAP scintigraphy he had splenic, renal and adrenal amyloid and had no evidence of progressive amyloidosis on serial SAP scans till census. During follow-up he had stable renal and hepatic biochemistry.

Discussion

A dominant hepatic presentation of systemic amyloidosis is relatively less common than dominant presentations associated with other amyloidotic organs. At the NAC over the 10 year period of this study 93/3348 (3%) new cases of systemic amyloidosis were diagnosed unexpectedly by liver biopsy. If hepatic amyloid is suspected clinically the current recommendations from the British Society of Gastroenterology are that diagnosis by percutaneous liver biopsy should be avoided as there is a risk of haemorrhage.[65] In the current series only 2/82 (2%) percutaneous liver biopsies were complicated by non-fatal haemorrhage.

Although abnormalities in liver biochemistry were present in all except 2 cases in the current series, it should be noted that such abnormalities can be unrelated to hepatic amyloid in patients with systemic amyloidosis. Alkaline phosphatase levels, for example, can be raised on the background of systemic inflammation. In a series of 374 patients with AA amyloidosis at the NAC one third had an elevated alkaline phosphatase but had no evidence of hepatic amyloid on SAP scintigraphy.[39] Cardiac failure, secondary to an amyloid cardiomyopathy, may also cause derangement of liver function tests and hepatomegaly through hepatic congestion. This can lead to elevations in alkaline phosphatase and aminotransferases, which typically tend to be relatively subtle.[224] Consistent with this in the current series some of the heredo-familial forms of systemic amyloidosis were diagnosed through liver biopsies of congested livers because of an isolated amyloid cardiomyopathy.

The vast majority of patients in the current series had AL amyloidosis. Extra-hepatic organ involvement, mostly in the heart and kidneys was present in most of these patients. Cardiac amyloid, in particular, had a significant impact on survival on multivariable analysis. Hypoalbuminaemia also impacted on survival and is a marker of both proteinuric renal dysfunction and also reduced liver synthetic function and is hence a surrogate marker of multisystem disease. The median survival of 1.2 years by Kaplan-Meier estimate from diagnosis of AL amyloid in the current series is likely to be an overestimate as several patients with hepatic presentations of systemic AL amyloidosis were too unwell to come for assessment at the NAC. Despite the poor prognosis historically associated with hepatic AL amyloid[35] there is supportive evidence in this study that, in the current era of chemotherapeutic regimes, in those who achieve a good clonal response to chemotherapy there is potential for amyloid regression from organs (Figure 8.1), for improvement of organ function and most importantly for a survival benefit to be conferred. This is even more apparent in those who had a >90% dFLC response to chemotherapy, who had a fivefold reduction in their mortality risk when compared to those who had a 50-90% dFLC response. There is a case therefore for chemotherapy to be administered to those who are fit enough to tolerate it with the aim of a quick and deep clonal response. With newer chemotherapeutic agents such as the proteosome inhibitors this could potentially be achieved with good effect.[80] The role of OLT in those patients with decompensated liver disease is unclear. It may prolong patient survival but from what little data is available in the literature this survival is poor compared to other liver diseases culminating in liver transplantation and in the current climate of donor shortages OLT in systemic AL amyloidosis is not a proven treatment strategy.[221]

A hepatic presentation of AA amyloidosis is relatively rarer with only 2 such cases in the current series over a ten-year period. In the series of 374 patients with AA

amyloidosis at the NAC followed till 2005 proteinuric renal dysfunction dominated, occurring in 97% cases, with a median survival in this group of 11 years.[39] Hepatic amyloid on the background of AA amyloidosis in a ^{123}I-SAP scintigraphic study at the NAC, negatively impacted upon survival.[42] In the context of AA amyloidosis it probably represents a large disease burden. However with adequate suppression of inflammation and maintenance of SAA values <10 mg/l, as occurred in one of the patients in this series, one can see amyloid regression from amyloidotic organs and stabilisation or improvement of organ function.[41]

Several hereditary forms of systemic amyloidosis were identified by liver biopsy in this series. Hepatic amyloid secondary to AApoAI occurred in 2 cases. Like AL amyloidosis, it is recognised as a cause of elevations in serum alkaline phosphatase and gamma-glutamyl transferase and also in causing decompensated liver disease.[225] Some of the other hereditary cases in the series had an isolated amyloid cardiomyopathy, which is indistinguishable from that of AL amyloidosis,[226] and had amyloid in the vasculature of their liver biopsies rather than parenchymal amyloid. The abnormal liver function tests that led to liver biopsies in these 3 cases were secondary to cardiac congestion because of a TTR-type amyloid cardiomyopathy (Patients 3, 4 and 5; Table 8.2). ATTR is not recognised as causing hepatic amyloid[29] and the diagnosis of systemic amyloidosis in these cases on retrospect was quite incidental and relied on the systemic nature of amyloid deposits throughout the vasculature. The cardiac failure secondary to a TTR-type amyloid cardiomyopathy may be more subtle than other forms of cardiac failure. There may be more dominant features of right-sided heart failure such as ascites and hepatomegaly.[226] Such a clinical presentation may prompt a clinician to suspect liver disease and hence pursual of a liver biopsy.

LECT-2 amyloidosis was discovered recently[227] and was identified unexpectedly in one case in the current series when a biopsy was pursued to investigate

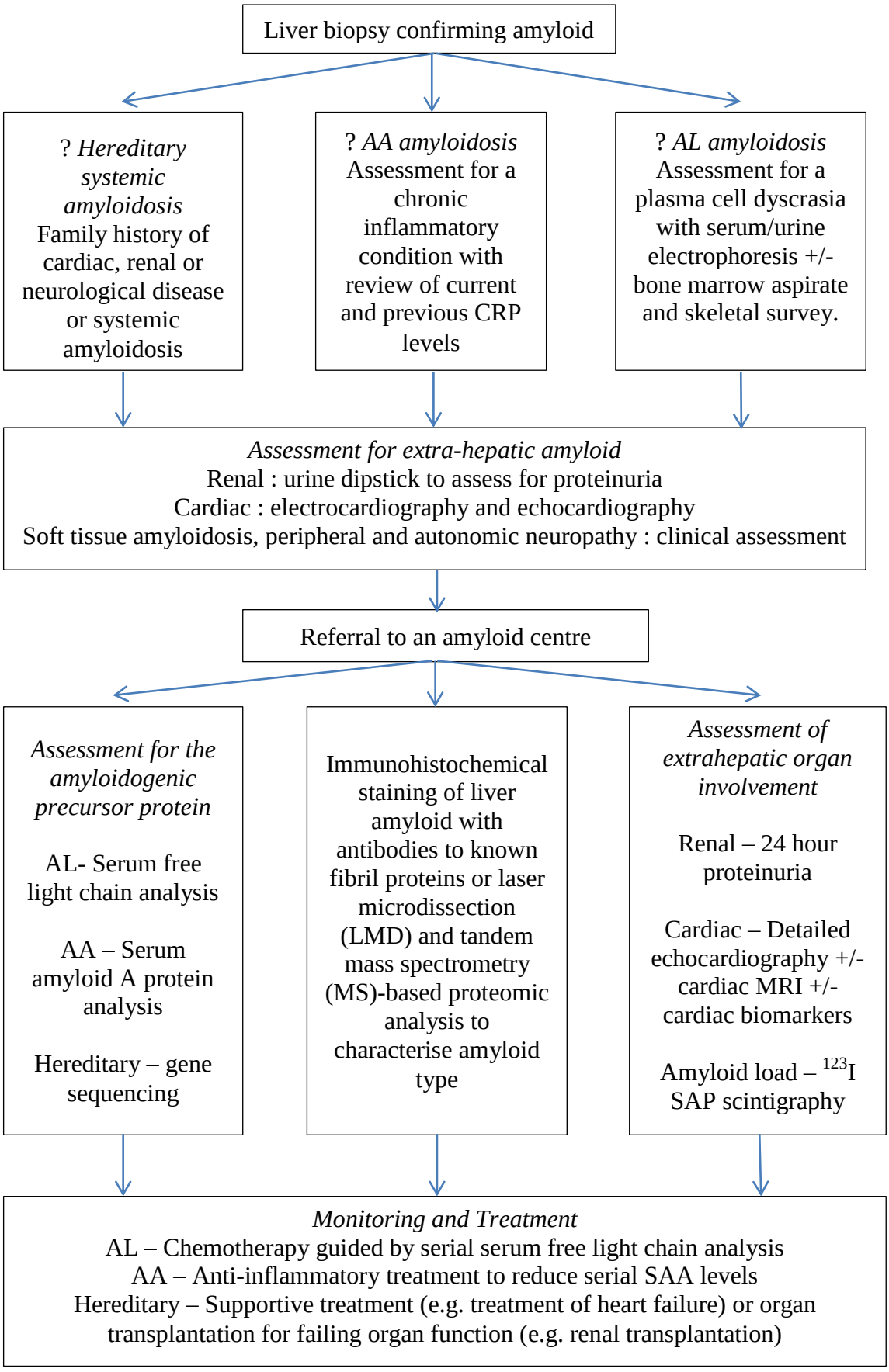
for potential fibrotic liver disease in the context of hepatitis C infection. It has also previously been diagnosed in 3 others cases with viral hepatitis, who have been followed at the NAC. In these cases it was characterised by hepatic and renal amyloid and slowly progressive organ dysfunction.[228] LECT-2 is thought to be a growth factor or chemokine.[227] It may have a role in liver regeneration and injury[229] and this could be a potential mechanistic link between viral hepatitis and this form of amyloidosis.

Any form of systemic amyloidosis can present with dominant hepatic features culminating in the diagnosis of amyloid by liver biopsy. AL amyloidosis is by far the commonest form in this setting. Correct diagnosis of amyloid type is imperative as prognosis and treatments vary significantly between these types. The hereditary amyloidosis syndromes, for example, have overlapping features with AL amyloidosis but whereas chemotherapy has a role in the latter it has no role in the former.[67] The difficulty in diagnosing hereditary amyloidosis is further compounded by the frequent absence of a family history, as the encoding mutations are often variably penetrant. As well as confirming amyloid type a comprehensive evaluation for extra-hepatic amyloid is also very important as it often co-exists with hepatic amyloid, impacts upon prognosis and often alters the approach to treatment. Figure 8.3 provides a potential algorithm for gastroenterologists in assessing patients with amyloid diagnosed by liver biopsy.

Conclusion

A hepatic presentation of systemic amyloidosis culminating in a liver biopsy when amyloid is not suspected is a relatively rarer presentation of systemic amyloidosis. Correct diagnosis of amyloid type is imperative as prognosis and treatments are vastly different between the different amyloid types. This mode of presentation is most commonly associated with AL amyloidosis but AA amyloidosis and heredo-familial

Figure 8.3. An algorithm for the typing of amyloid confirmed on liver biopsy and the subsequent assessment of systemic amyloidosis.



forms of amyloid can also present in this manner. In the setting of AL amyloidosis this presentation confers a poor prognosis, especially when there is concomitant cardiac amyloid, hypoalbuminaemia or hyperbilirubinaemia. However in those with a good clonal response to chemotherapy a survival advantage can be conferred.

General Conclusions

The studies in this thesis have highlighted several novel findings concerning the phenotype, investigation and management of the systemic amyloidosis syndromes.

The role of solid organ transplantation appears to be differentially beneficial depending upon which organ is transplanted and in which form of systemic amyloidosis. Liver transplantation, as a form of surgical gene therapy, is the only available treatment for familial amyloid polyneuropathy. Most of its evidence relates to the V30M TTR variant. FAP T60A, unlike the well-described FAP V30M, is characterised by a dominant cardiac phenotype rather than neuropathy, which appears to have a significant bearing on survival in affected individuals in a similar manner to the impact of cardiac amyloid in patients with AL amyloidosis. Therefore careful evaluation for cardiac amyloid with echocardiography and cardiac biomarkers is warranted in this patient group to inform on prognosis. Whereas liver transplantation can have a significant impact upon disease progression and survival in FAP V30M its role appears to be less compelling in those with FAP T60A and again this appears to be related to cardiac amyloid, which can act as a nidus for an accelerated amyloid cardiomyopathy secondary to wild-type transthyretin synthesised in the liver graft in the post-transplant setting. An unexplored area in TTR-related cardiac amyloid is the role of cardiac biomarkers. A possible extension from the study of NT-proBNP in this thesis would be the role of serial NT-proBNP assessment in FAP patients, which has been found to be of prognostic significance in patients with AL amyloidosis. The role of cardiac MRI, which is a newer investigative modality, in the assessment of these patients could also potentially provide prognostic information.

Solid organ transplantation appears to be more successful when used to replace failing amyloidotic organ function in the other forms of systemic amyloidosis. ALys,

unlike other hereditary forms of systemic amyloidosis appears to be associated with mutations that are very penetrant and as a result affected individuals inevitably have a family history of associated features such as renal failure and hepatic rupture. RTx and OLT for these respective complications appears to be successful with good graft and patient survival. RTx also appears to be a feasible option in patients with AA amyloidosis and ESRD associated with IBD, provided that good inflammatory control, guided by serial SAA levels, is maintained. In those with organ failure on the background of AL amyloidosis, RTx in those with a good ECOG performance status, isolated ESRD and a pre-transplant clonal response to chemotherapy and cardiac transplantation in those with isolated end-stage cardiac disease followed by SCT appears to be feasible with good graft and patient survival. OLT in those with decompensated liver disease has poor graft and patient survival outcomes compared to OLT in other liver diseases. Whereas those with end-stage cardiac disease are generally too unwell to receive any treatment of their clonal disorder before cardiac transplantation timing of treatment of an underlying clonal disorder relative to renal transplantation in this relatively fitter cohort is less clear-cut. There is good rationale to treat the clonal disorder before renal transplantation to try and prevent amyloid recurrence in the graft but there are also potential advantages in performing transplantation first as the subsequent normal renal function would preclude the need for dose attenuation of chemotherapy and hence this would offer the best chance of a deeper clonal response and hence also potentially reduce the likelihood of graft amyloid recurrence in the longer term. Studies to compare these two approaches are therefore warranted.

There have been few studies of hepatic and gastrointestinal amyloid. From studies in this thesis it would appear that in ALys hepatic amyloid is virtually universal at presentation, although liver biochemistry is rarely abnormal. It is apparent from the

liver biopsy study that diagnosis of amyloid unexpectedly in this way in those with abnormal liver biochemistry and/or hepatomegaly is a rarer mode of presentation of systemic amyloidosis. It is most commonly associated with AL amyloidosis, which in this setting appears to have a worse prognosis than other forms of amyloidosis presenting in a similar manner. It can be concluded that correct diagnosis of amyloid type is imperative as both prognosis and treatments are very different between these types. Awareness of the multisystem nature of the presentation of those with AL amyloidosis in a liver biopsy and assessment for potential extra-hepatic amyloid is extremely important as this confers a mortality risk. AL amyloidosis with a dominant liver presentation has historically been associated with a poor prognosis but it would appear that if a good clonal response can be achieved with chemotherapeutic regimes in the modern era of treatment a survival advantage can be conferred. This benefit could be further accentuated with newer agents such as the proteosome inhibitors and studies of these agents are required.

Gastrointestinal symptoms secondary to amyloidosis may occur in the context of intestinal infiltration by amyloid, as is common in ALys which is associated with a high frequency of endoscopic abnormalities or be associated with an amyloidotic autonomic neuropathy, as probably occurred in the FAP T60A cohort. Detailed evaluation to corroborate clinical suspicion of gastrointestinal dysmotility in this latter group has not been systematically studied and warrants further research with techniques such as gastric scintigraphy, radio-opaque marker assessment of colonic transit, wireless capsule motility studies, anorectal manometry and testing for small intestinal bacterial overgrowth, which can be associated with gastrointestinal dysmotility. Systemic amyloidosis can cause an array of GI complications but, conversely, primary diseases of the gastrointestinal tract can cause amyloidosis. IBD is one of the commonest causes of AA amyloidosis. Its major mode of presentation is proteinuric renal dysfunction. Early

diagnosis with urine dipstick is therefore very important and should prompt consideration of the diagnosis. In those with well controlled inflammation, guided by serial SAA levels rather than CRP levels, one can see improvement of amyloidotic organ dysfunction. There needs to be awareness of the risk of decompensation of amyloidotic kidney function in the face of physiological stresses such as hypotension associated with sepsis and instigation of measures to prevent this from happening.

The study of nutritional status in AL amyloidosis is one of the largest prospective studies in systemic amyloidosis, performed to date. Malnutrition is prevalent, particularly in the context of multisystem disease and is not only associated with a poor quality of life but is also independently associated with reduced survival. It therefore provides another potential target for intervention studies, which have to date largely focused on chemotherapeutic strategies. One potential intervention would be dietary advice and nutritional supplementation, which could be set up in the form of a randomised study. Another potential area of research would be into the mechanisms of cachexia in AL amyloidosis with a focus on potentially causative cytokines such as TNF-α, IL-1 and IL-6 which could provide a pharmacological target.

Publications arising from the thesis

Sattianayagam PT, Hawkins P, Gillmore JD Systemic amyloidosis and the gastrointestinal tract. Nat Rev Gastroenterol Hep 2009; 6(10): 608-617

Sattianayagam P, Hawkins P, Gillmore J Amyloid and the gastrointestinal tract. Expert Rev Gastroenterol Hepatol 2009; 3(6): 615-630

Sattianayagam PT, Hawkins PN (2012) Systemic amyloidosis. (in press)
Textbook of Clinical Gastroenterology and Hepatology (Second Edition - edited by Christopher Hawkey, Joel Richter, Jaime Bosch, Guadalupe Garcia-Tsao and Francis Chan)

Sattianayagam PT, Gibbs SDJ, Pinney JH, Wechalekar AD, Lachmann HJ, Gilbertson JA, Hawkins PN, Gillmore JD Solid organ transplantation in AL amyloidosis.
Am J Transplant 2010; 10 (9): 2124-2131

Sattianayagam PT, Hahn AF, Whelan CJ, Gibbs SD, Pinney JH, Rowczenio D, Stangou AD, Pflugfelder PW, Fox Z, Lachmann HJ, Wechalekar AD, Hawkins PN, Gillmore JD Cardiac phenotype and clinical outcome of familial amyloid polyneuropathy associated with variant transthyretin (T60A).
Eur Heart J 2012 (epub ahead of print)

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J Intern Med 2011 (epub ahead of print)

Sattianayagam P, Gibbs SD, Wechalekar AD, Gilbertson JA, Lachmann HJ, Hawkins PN, Gillmore JD Systemic AA Amyloidosis in Crohn's Disease (CD): A Serum Amyloid P Component (SAP) Scintigraphy Study.

Gastroenterology 2009; 136 (5) Supplement 1: A188

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Gastroenterology 2010; 138 (5) Supplement 1: S366

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Gastroenterology 2011; 140 (5) Supplement 1: S864

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Appendices

Appendix 1

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’

Appendix 2

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Date

Dear Haematologist, GP, referring doctor

Re. Patient details

We are trying to gather some information on the above patient here at our Centre. Below are some questions and we would be very grateful if you would be able to provide us with as much information as possible. We would be very grateful if you would kindly forward any relevant correspondences to us or failing that annotate on this letter and send it back.

Did the patient receive any chemotherapy, and if so what regimen and how many cycles?

Was there a response, and if so was it a partial or complete response?

Was he/she hospitalised at any point and if so for how many days?

If he/she was admitted to hospital, what was the reason for admission?

Did he/ she receive any antibiotics for infections? If so what was the infection and how long were antibiotics given for?

Is the above patient dead and if so when and what was the cause of death?

Thank you very much for your time and assistance.

Yours sincerely

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